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SURGICAL TREATMENT OF ANGINA PECTORIS

REPORT OF EIGHT ADDITIONAL CASES AND REVIEW OF LITERATURE

PHILIP KING BROWN, M.D.

AND

WALTER B. COFFEY, M.D.

SAN FRANCISCO

When we undertook to relieve angina by following the suggestion of Franck as carried out first by Jonnesco,[1] we were fully conscious that all that had been accomplished by Jonnesco's first operation was to relieve the substernal anginal pain and the associated referred pains in his patient, by a removal of the left cervical sympathetic system and the upper thoracic ganglion. The functions of much of the removed parts were the subject of some important differences of opinion, and certainly many tracts transmitting a variety of impulses were severed in Jonnesco's operation. It seemed to us wise, therefore, to simplify if possible this complex situation by beginning at one end of the tract and severing as few connections as possible, continuing to interrupt paths between heart and brain and cord until we found just what brought the relief.

Our first four patients[2] were operated on by severing merely the main left sympathetic trunk below the superior ganglion, and the superior cardiac branch. In the fifth patient the ganglion was removed, because the superior cardiac trunk arose in part from that by at least one and we thought possibly by several roots. Besides this, the fourth patient had had pain recur in the left forearm, although the substernal pain had been relieved. That other branches through vagus connections might be of influence in relaying sensation seemed possible, and the consequences of a removal of the ganglion seemed insignificant as compared to the incomplete relief of pain. Since that time, the superior

1. Jonnesco, T.: Angine de poitrine, guerie par la resection du sympathique cervico-thoracique, Bull. de l'Acad. de méd., Paris **84**:93 (Oct. 5) 1920; Le traitement chirurgical de l'angine de poitrine, ibid. **86**:67 (July 19) 1921; Traitement chirurgical de l'angine de poitrine par la resection du sympathique-cervico-thoracique, Presse méd. **20**:193 (March 9) 1921; La resection du sympathique cervico-thoracique; technique operatoire, Presse méd. **30**:353 (April 26) 1922.

2. Coffey, W. B., and Brown, P. K.: The Surgical Treatment of Angina Pectoris, Arch. Int. Med. **31**:200-220 (Feb.) 1923.

ganglion has always been removed. One patient (Case 3) of the first group has been operated on a second time, after the failure of section of the main trunk and superior cardiac branch to relieve permanently the pain referred to the region of the heart apex and left arm, and the occurrence of new pain in the back behind the heart in the vicinity of the angle of the left scapula. That case is not counted in the new group, although it will be discussed as Case 9 in the light of the further relief of the patient's pain after the second operation, in which the superior ganglion was removed after merely the severance of its main trunk and the superior cardiac connections in the first operation. Case 8 [3] was reported in full and it contributes one more fact to the slowly accumulating data regarding angina, and heart and aorta innervation. In that case the pains were substernal but the radiations were dextral and a right-sided operation, in which the superior ganglion was removed, caused complete relief from attacks.

Of this group of additional cases, one (Case 1) patient died a cardiac death within a few hours of the operation and another patient (Case 5) in two weeks, also of heart failure. Both had low blood pressure, large flabby hearts and negative T waves in Lead I. The pains in both were substernal, but also in the apex region as well as down the left arm. In Case 1 the death of the patient was unexpected and hard to explain. In Case 5 the death of the patient was not unexpected. He had consumed alcohol and opiates to excess for years, was extremely obese, and had a chronic cough and an extreme dyspnea at the time of operation. The attacks were so numerous and severe that he was in pain day and night, and the operation was recognized as a desperate chance.

The other operations were brilliant successes, so far as relief of the agonizing substernal pain of angina goes. The patients were all restored to comfort and some degree of usefulness, depending on the underlying heart condition. One patient still has severe pain for a second or two in the angle of the left jaw when he begins to chew food, and he and one other have had pain in the posterior part of the left apical region above the center of the scapula. There is not only pain on pressure but pain when the shoulder is raised from extension of the left arm at right angles to the body. These pains in jaw and left shoulder have occurred, to some degree, after six of the fourteen operations done (on thirteen patients) and have been of minor consequence, disappearing in a few weeks in all but two patients. The jaw pain seems to be due to pressure from retraction on the anterior auricular nerve, and the left posterior shoulder region pain must be due to injury of some connection of the nerve and ganglion.

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3. Brown, P. K.: Cervical Sympathectomy for Angina Pectoris: Report of a Case with Dextral Radiations of Pain, J. A. M. A. **80**:1692-1693 (June 9) 1923.

Two patients (Cases 6 and 9) have had pain in the region of the heart apex, and in the region of the angle of the left scapula and down the left arm.

Whether we have gone too far in removing the superior ganglion was and is still a matter of some doubt. Important light on the subject is to be looked for in one patient, in whom pain returned after one and one-half years, and whose pain was apparently relieved by removal of the superior ganglion which was not removed at the first operation (details given in Case 9). If the patient continues free from pain under present conditions, there must be connections between heart and spinal cord that were interrupted by this procedure. Should the pain return again in the region of the heart apex and in the arm, it is still possible to do one of two things, remove the two lower ganglions in the sympathetic chain and the upper thoracic or cut the sensory roots in the spinal canal.

It must not be forgotten that complete relief of the main and severest pain, and to our minds the most serious symptom of angina, came from the operation of cutting only the main sympathetic trunk and the superior cardiac nerve on the left side. In the light of this, one fact stands out clearly, that the substernal pain is a spasm of the first part of the aorta where the superior cardiac branch is largely distributed, and it is the vasoconstrictor influence of this nerve, not only to the aorta but the coronaries which it also supplies, that is responsible for the main symptom of angina. Death occurs when this constrictor action on both aorta and coronaries deprive the heart of blood, and this effect is contributed to by sclerosis of both the aorta and the coronary vessels. It is possible that constrictor impulses are relayed to some extent by the middle ganglion, which, according to some authors (Figs. 1 and 2) may give off a branch to the superior cardiac nerve below where it was cut in our operation. This would account for the persistence of certain referred pains in a few of the cases.

There is one other point that needs consideration, and on which light can only come from developments in patients already operated on, and that pertains to the work of Wenckebach,[4] and Eppinger and Höfer in Vienna. Wenckebach reported five cases in which the depressor nerve was resected on the left side. This nerve, according to Krielman, arises by two roots, one from the superior laryngeal and the other from the vagus. These two join, forming a cord which soon unites with the vagus. Finkelstein[5] found this branch in only two of five cadavers. Békésy found it in nine out of fourteen cadavers, and states that it runs isolated for only 1 cm. and then joins the vagus again. It exists as a

4. Wenckebach, K. F.: Deutsch-Internisten Kongress, Vienna, April, 1923.
5. Finkelstein: Der Nervus depressor beim Menschen, Kaninchen, Hunde, bei der Katze und dem Pferd, Arch. f. Anat. u. Phys. Anat. Abt., 1880, p. 245

separate nerve in animals according to von Cyon.[6] The existence of a separate depressor nerve in man is denied by American physiologists and anatomists, so far as we have been able to carry our search. Odermatt [7] reviews the literature on this nerve, and describes an attempt to cut it in a case of bad angina. Hotz of Basel operated under local anesthesia on a man, aged 65 years, who had had scarlet fever and rheumatism in youth. A diagnosis of tetany and syphilis was made

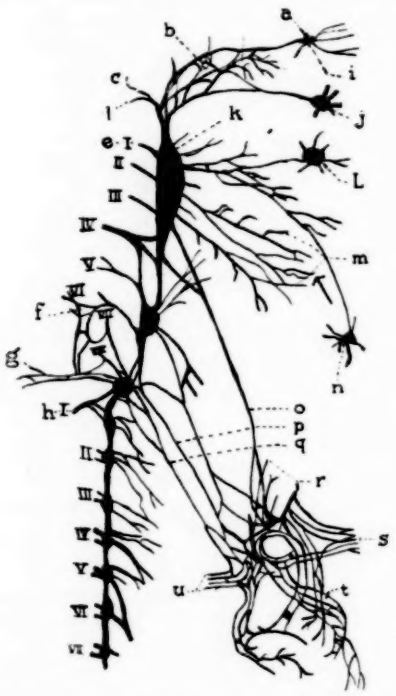

Fig. 1.—The cervical and dorsal sympathetic systems, with connections to spinal cord and vagus. *a*, cavernous plexus; *b*, internal carotid plexus; *c*, ramicommunicantes between gangliated cord and *d*, ganglion jugulare; *e*, cervical nerves I, II, III, IV, V and VI; *f*, plexus about vertebral artery VII; *g*, plexus about subclavian artery VIII; *h*, thoracic nerves I, II, III, IV, V, VI and VII; *i*, cilary ganglion; *j*, sphenopalatine ganglion; *k*, ganglion nodosum vagus; *l*, otic ganglion; *m*, connections with vagus and glossopharynx to form pharyngeal plexus (inhibitory); *n*, submaxillary ganglion; *o*, *p* and *q*, superior, middle and inferior cardiac nerves (accelerator); *r*, connections with vagus and recurrent laryngeal nerves (inhibitory); *s*, left pulmonary plexus; *t*, cardiac plexus; *u*, right pulmonary plexus.

6. Von Cyon: Die Nerven des Herzens, Ubersetzt v. Heusner, Berlin, J. Springer, 1907.

7. Odermatt, W.: Deutsch. Ztschr. f. Chir. **182**:341 (Sept.) 1923.

in 1921. For four years he had had cramps in the hands and stasis in the veins. The Wassermann test was positive. Blood pressure at this time was 170. An antisyphilitic treatment left him free from trouble for one and one-half years. Following this, he began to have pressure in the heart region, air hunger, anxiety, and pain in the right shoulder. In the hospital he continued to have attacks more and more frequently, in spite of much rest in bed and general treatment. The pains radiated to the left shoulder blade and arm, as many as four attacks occurring in one day.

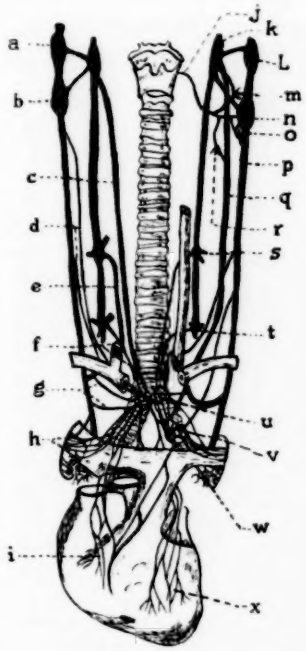

Fig. 2.—Cardiac, pulmonary and coronary plexuses; *a*, ganglion of root (inhibitory); *b*, ganglion of trunk (inhibitory); *c*, superior cardiac nerve (accelerator); *d*, cervical cardiac branches of vagus (inhibitory); *e*, middle cardiac nerve (accelerator); *f*, inferior cardiac nerve (accelerator); *g*, thoracic-cardiac branches of vagus (inhibitory); *h*, right inferior pulmonary plexus; *i*, right coronary plexus; *j*, superior laryngeal; *k*, superior cervical ganglion (accelerator); *l*, ganglion of root (inhibitory); *m*, branch from superior cervical ganglion; *n*, ganglion of trunk; *o*, depressor; *p*, left vagus (inhibitory); *q*, superior cardiac nerve (accelerator); *r*, branch of superior laryngeal to superior cardiac; *s*, middle cervical ganglion (accelerator); *t*, inferior cervical ganglion (accelerator); *u*, deep cardiac plexus; *v*, superficial cardiac plexus; *w*, left posterior pulmonary plexus; *x*, left coronary plexus.

The incision was made along the sternocleidomastoid muscle and the relations shown in Figure 3 were made out.

Stimulation by faradic current of the vagus caused severe pain in the breast, faradization of the fibers known as the depressor caused slight cough but no alteration of the pulse. Blood pressure varied from 120 to 145 systolic and from 75 to 105 diastolic, mounting steadily toward the end.

Following the operation, the patient had dyspnea and complained of air hunger. Edema developed; the heart dilated. There was no further angina. He died fourteen days after the operation with heart insufficiency symptoms.

Odermatt is not certain what was cut in the operation.

Fig. 3.—*a*, sympathetic of neck; *b*, hypoglosseal; *c*, branch of hypoglosseal; *d*, vagus; *e*, laryngeal superior; *f*, and *g*, the two roots of depressor.

Dr. S. W. Ranson (Northwestern University), whose studies and comments on this work have been most helpful, is of the opinion that the good results in Wenckebach's [4] patients were due to the fact that Hofer, who operated on them, cut the superior cardiac branch thinking it was the depressor. There is still one other theory worth setting forth, and that is that there are connections between the superior cervical ganglion and the two ganglions of the vagus, as well as with the superior laryngeal, and in removing the cervical ganglions some important bypaths are interrupted which contribute to stabilizing the first part of the aorta and the coronaries. This does not make the situation any less complex, and we will now review the operations on angina published to date and

reported to us by letter, in the final attempt to show that the chief result in all the proposed and executed operations has accomplished only what has been accomplished in a large majority of our cases and patients operated on by our procedure.

REVIEW OF THE LITERATURE

The operative procedures to date are divided by Kappis [8] into five groups.

1. Resection of the middle and lower cervical and upper thoracic sympathetic ganglions on the left side. Three operations, two by Jonnesco [1] in syphilitic men, aged 38 and 54, respectively, and one by Tuffier [9] in an arteriosclerotic man, aged 50. In all, the anginal attacks were relieved.

2. Resection of all three ganglions of the left cervical sympathetic chain and the upper thoracic ganglion. Brüning,[10] Kummell and Kappis [8] each operated on women, aged about 60 years. Brüning and Kummell's cases were rendered free from attacks. Kappis' case, a woman, aged 62, with increasingly severe attacks of pain in the heart region and left arm, whose blood pressure was 200 systolic and 170 diastolic had an attack during the operation. Four months after operation, after exertion, attacks of pain began again behind the lower sternum and in the back. Accompanying this also was a feeling of anxiety.

In this connection must be mentioned a brief report by Pleth of Stockton, who claims to have done the Jonnesco operation on four patients with angina, with relief of pain. One died of aspiration pneumonia. The published report is without detail.

3. Resection of the main trunk and superior cardiac branch of the superior cervical ganglion. Five cases were reported by us, one patient died six hours after operation.

4. Severing of the "depressor vagi." Eppinger in Wenckebach's clinic reports five patients operated on by Hofer, two on the left side alone, and three on both sides; one died and the others were relieved. Odermatt [7] reports a sixth patient who died in thirteen days of progressive heart insufficiency.

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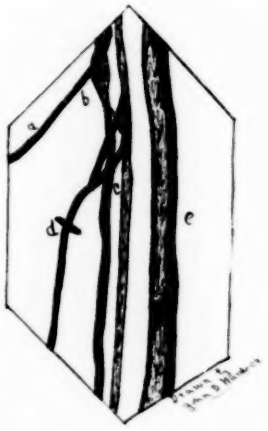
8. Kappis, M.: Diskussionsbemerkungen zu, Das Schmerzproblem der Eingeweide, XLVI Kong. d. deutsch. Gesellsch. f. Chir., 1922, Arch. f. klin. Chir. **121**:188, 1922; Med. Klin. **19**:1658 (Dec. 23) 1923.

9. Tuffier, T.: Deskussionsbemerkung, ref. Bull. de l'Acad. de méd., Paris **86**:99 (July 26) 1921.

10. Brüning, F.: Die operative Behandlung der Angina Pectoris durch Exstirpation des Hals-Brustsymathicus und Bemerkungen uber die operative Behandlung der abnormen Blutdrucksteigerung, Klin. Wchnschr. **2**:777 (April 23) 1923; Arch. f. klin. chir. **126**:490, 1923.



Fif. 4.—Site of operation. *a*, laryngeal superior; b, sympathetic nerve; *c*, vagus; *d*, depressor; *e*, carotid.

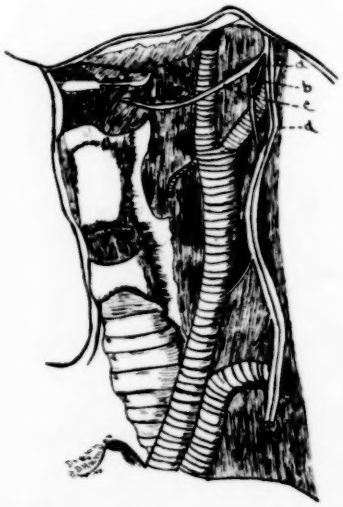


Fig. 5.—*a* and *b*, two roots of depressor nerve; *c*, superior laryngeal; *d*, vagus.

5. Severing of the "depressor vagi" and extirpation of the lower half of the left superior ganglion to the lower half of the middle ganglion. Borchard [11] reports such an operation on a man, aged 54, who, except for a light attack on the fifth postoperative day, was relieved. He died, three weeks later, of acute circulatory disturbance in the brain.

To these groups should be added a case reported by us (Case 2) of dextral radiations of pain, in which a resection of the *right* superior cervical ganglion was done with complete relief.

6. We now add nine cases, in which the operation consisted in removal of the superior ganglion, one of them just referred to and one where the ganglion removal followed a year and a half after simple section of the left trunk and superior cardiac branch. Of these one patient died as a result of the operation and one two weeks later. The others have been relieved, except Case 6, and he has been relieved of all but the referred pain and pain due to trauma of operation.

To this group are added brief reports of cases by Bacon, Holmes, Naffzinger, Lambert, Marvin (two cases) and Mortensen. The patients were all relieved of the substernal agony and with but one disturbing complication (Marvin's first case). Other successful operations have been done but the results were not published, and reports of them were not available to us.

REPORT OF CASES

CASE 1.—H. S. H., a man, aged 54, entered the hospital on Sept. 19, 1922. The entrance diagnosis was angina pectoris.

Present Illness.—The patient had lower third substernal discomfort and some shortness of breath for the last few years. For the last few months the condition had become more serious. On the least exertion he had pain in the center of the chest and epigastrium, which eating exaggerated at times (from one-half hour on). He did not vomit, but belched at times. The pain radiated to shoulders and arms, and he had numbness and tingling in the hands. The pain passed away in from five to ten minutes, and went faster if he assumed an erect position. The attacks of "distressing pain" under the lower third of sternum began fully two years ago. They occurred as an incident to excitement or muscular effort, and consisted of a painful feeling of pressure, which would extend upward across the upper chest and into the back between the shoulder blades, and recently down the arms where it consisted of an ache in both arms and upper forearms. There was also numbness, tingling and pain in the fingers, but no neck pain. The pain was equally distributed to the two sides. These attacks would come four or five times a day, growing more frequent as time went on, and following less exertion. Once in a while he awoke with an attack the usual duration of which was from five to ten minutes. Belching might relieve an attack, and soda seemed to help. A nitroglycerin tablet relieved in a few minutes. Standing up seemed to help always. The attacks did not vary much in severity or duration. Shortness of breath marked the condition in the beginning. The patient lost 25 pounds (11.3 kg.) in the last three months. His average weight for twenty years was 210 pounds (94 kg.). He dieted last May and lost 20 pounds (9.0 kg.). In April, 1922, the attacks became

11. Borchard, quoted by Kappis, Footnote 8.

more severe, the pain extending down the arms for the first time. The first severe attack followed running for a car. The patient had no sense of "impending death" in any attack.

In spite of diet and nitroglycerin and more control of effort, attacks continued. Shortness of breath did not bother him recently.

Family History.—Negative.

Past History.—He had not smoked for two months. He ate regularly and his appetite was good. He ate only two meals a day, and slept fairly well. The bowels were normal. He had nycturia three years ago, and had had measles and mumps. He did not have malaria or typhoid. The patient denied venereal history. He has had no rheumatism or gout and never underwent an operation. He has never had any injuries.

Physical Examination.—The general appearance was of a well nourished man, with rosy color, the size and shape of whose head was normal, the eyes clear, pupils equal and regular and reacted normally. The nose was normal. The hearing was good. The mouth and teeth were fair, some teeth were crowned, the membranes were normal and the throat negative. Partial tonsils were still evident. In the chest the breath sounds were roughened.

Heart.—Blood pressure was 108 systolic, 62 diastolic, the sounds were not loud owing to the thick chest wall, there were no discrepancies and no irregularity; the pulse was full, regular and compressible.

Abdomen.—There were no masses, tenderness or rigidity. The genitalia were normal.

Reflexes.—The knee jerks were normal. On September 20, the blood pressure was 108 systolic, 62 diastolic. September 22, the patient had no pain since he entered hospital because he has been kept (1) at rest; (2) on light diet; (3) on digitalis and sodium nitrite for the last twenty-four hours.

The patient recognized the connection between his pain and physical effort. The nitrite was stopped on account of the absence of pain and low blood pressure, 108 systolic, 60 diastolic.

Urinalysis.—Amber; clear; specific gravity, 1.015; alkaline; no albumin; no glucose. Microscopic examination showed very few amorphous phosphates, few red blood cells and mucous threads.

Gastric Contents.—Test meal. The amount obtained was 105 c.c., normal in character, the total acidity of which was 59, free hydrochloric acid 52.

Wassermann Report.—Negative.

Fluoroscopic Examination.—Showed a thickened dilated aorta. The aorta almost filled the postcardiac space. There was considerable hypertrophy of the left ventricle.

September 23, the pulse was 54. Blood pressure, 114 systolic, 60 diastolic. The patient had slight pain before breakfast in the morning, which lasted for a few seconds. September 25, the patient was still on digitalis. The pulse was 68. There was no pain. The blood pressure was 114 systolic, 56 diastolic. September 26, roentgenograms, taken from the lateral and from the anteroposterior, showed considerable thickening of the aorta, also some dilatation. Both diaphragms were irregular, especially the right. Heavy peribronchial thickening, also hilum thickening was seen in the chest.

Conclusions.—Considerable radiologic evidence of aortitis.

September 27, the patient had no pain and was given one week's leave. October 4, he returned in the morning. He noted slight pain whenever he made any special exertion, such as taking a three-block walk, or went upstairs at all fast or carried a grip.

The patient woke up with pain at midnight a few nights before; he had eaten a fairly good dinner. He was not constipated. His weight the morning of return was 182 pounds (82.6 kg.), a gain of 2 pounds (0.9 kg.) in a week. The blood pressure was 111 systolic, 56 diastolic. The pulse was 72.

October 5, electrocardiogram showed the T wave inverted in Lead I. The vital capacity was 3,760 c.c., about 80.5 per cent. of the normal height standard.

October 6, the weight was 181. The patient had pain in the morning, caused by rubbing himself with a bath towel. The pain passed away when he stood up. The blood pressure was 122 systolic, 64 diastolic. The pulse was 62 and regular. The patient was taking no medication.

October 11, operation was performed for the resection of branches (cardiac and trunk) of the superior cervical ganglion, after ⅙ grain morphin, $\frac{1}{150}$ grain atropin, nitrous oxid and ether anesthesia. The duration of the operation was forty-five minutes.

The pulse was 88, respiration 20, the patient's color was good. The pupils were contracted. He was perfectly conscious one hour later, and complained of pain in the chest two and one-half hours later. The pulse just before this was good. The patient vomited five minutes later and appeared to be choking. The intern administered epinephrin and later camphorated oil, followed at once by oxygen as the color was pale and the pulse imperceptible. The patient died a few minutes later, twenty minutes after the vomiting began. Necropsy was refused.

This was regarded as a cardiac death, and believed to be referable to serious coronary sclerosis and myocardial disease.

Case 2.—J. D., a man, aged 68, entered hospital with marked cardiac decompensation, auricular fibrillation and dyspnea, after three months of care at home following a severe attack of angina.[12] Several mild attacks a day were the rule, and in the three months interval three severe attacks had occurred.

The pain was under the upper sternum and the radiations were dextral, except in the first attack where they extended to both arms. The right superior cervical ganglion was removed by Dr. W. B. Coffey, with complete cessation of both the severe and minor attacks.

The negative T wave in Lead I before operation was not visible after the operation.

Case 3.—P. J. M., a man, aged 41, Danish, a boiler-maker, was admitted to the Southern Pacific Hospital, April 23, 1923. The diagnosis was angina pectoris, with the complication of aortitis.

Family History.—Negative.

Past History.—Negative.

The patient was married and had one child. He denied venereal history.

Complaint.—The patient had pain over the heart on slight exertion for the last two and one-half months. He got wet while at work, and had a mild form of influenza on February 7, and immediately afterward he noticed shortness of breath, and a few days later noticed pain in the precordium after exertion. In two weeks the pain was severe enough to confine him to bed. This pain first started just under the left of the sternum about at the level of the eighth rib, and immediately traveled up to the neck and out both arms, stopping at the elbow usually, and always on the inside of the arm. This pain in the first attack lasted for about thirty-six hours. Since that he has had attacks lasting for only a few minutes. The pain in these attacks did not travel down the arms but did go up to the neck. These minor attacks came on after eating heavily, slight exertion and excitement.

For two months (since February 16) he had been laid up. He had what he called "flu." His average weight was 206 pounds (92 kg.) now 185 (83.9 kg.).

Physical Examination.—The patient appeared healthy in all respects, except for a purplish flush on his face, which was most noticeable on the lips. The head showed no abnormalities. Blood pressure, 125 systolic, 85 diastolic. The

12. Brown, P. K.: Cervical Sympathectomy for Angina Pectoris: Report of a Case with Dextral Radiations of Pain, J. A. M. A. **80**:1692-1693 (June 9) 1923.

pulse was slow, 66, regular and appeared normal. The heart was slightly enlarged, the tones faint, with slight systolic roughness over the aorta. There were no irregularities of rhythm.

The abdomen was negative. The extremities were normal. The reflexes, normal.

April 24, the blood pressure was 100 systolic, 76 diastolic.

Urinalysis.—Negative.

Blood Report.—Hemoglobin 90. Erythrocytes, 5,040,000. Color index 9. Leukocytes, 5,000.

April 26, the Wassermann test was negative. Vital capacity, 4,050, 96 per cent.

Roentgen-Ray Examination of Heart and Aorta.—The entire left chest was obscured by a faint opaque shadow. Interfissural thickening had taken place on the right side. The aorta was broadened. There was general hypertrophy of the heart muscles.

Conclusion.—Arteriosclerosis of the thoracic aorta, and general hypertrophy of the heart muscles. The opaque shadow seen on the left side could be due to congestion; however, it was more reasonable to believe that a chronic pleuritis had taken place on this side.

May 3, the patient still had some pains. There was a subacute inflammation of the entire vocal box. There appeared to be some inhibition in movement of the left vocal cord.

Electrocardiagram showed left ventricular preponderance and some indication of a negative T wave in Lead I.

May 17, the patient was dismissed somewhat improved.

June 11, he was readmitted to hospital, after two weeks' leave, because of persistent attacks of pain and inability to work.

The patient stated that since he left the hospital he has had daily attacks of pain in the precordial region, without any radiation. During these attacks he felt as if he were suffocating.

June 12, the blood pressure was 134 systolic, 96 diastolic.

June 21, the patient was taken to the operating room at 9:30 a. m. The anesthetic of nitrous oxid and ether was started immediately. When the patient came to the surgery he was in a highly nervous state. At 9:40 he began to go into what appeared to be a collapse. His pulse became very weak, in fact hardly obtainable and irregular. We were unable to hear any heart sounds. His breathing became very irregular, slow and shallow. The color of his face became very peculiar, the left side was blue in one portion and pale in another, there being quite a marked distinction between these two areas. The right side of the face had a grayish pallor. He looked like a dying man.

The anesthetic was stopped, and 3 c.c. of camphorated oil was given him hypodermically. In about five minutes his condition became better, the heart and pulse were fairly good, and the ether anesthetic was continued.

The operation was begun at 10:05 and ended at 11, with the closing pulse 84, respiration 20 and the color fairly normal. The left superior cervical ganglion was removed by Dr. Coffey.

June 22, the patient complained of sore throat and spoke in a whisper. He had a temperature of 101. The pulse was 100.

June 23, he had no pain in the precordial area or shoulder since operation, and said he felt fine. He still complained of sore throat. The temperature was 101. The patient was kept on a liquid diet.

June 24, he was able to speak a little louder. The sore throat was still present.

June 25, the patient spoke better. The sore throat was disappearing. Blood pressure, 130 systolic, 92 diastolic, in both arms.

July 2, he had a desquamative dermatitis on the right leg before operation, and this spread all over his body and on the fingers and palms, not on his feet

or face. He complained of numbness in the left side of the face, hoarseness and cough, and said his tongue was in his way and that he could not chew without biting it. The blood pressure was 110 systolic, 80 diastolic.

July 9, the patient had no pain whatever and felt better than at any time since the operation. The hoarseness continued, also the difficulty with the tongue. The cough was better. The dermatitis was practically gone. The blood pressure was 135 systolic, 110 diastolic, and the patient was discharged.

August 17, he was readmitted to the Southern Pacific Hospital, with the admission diagnosis of brachial neuritis (traumatic). The patient was given hot magnesium sulphate compresses, with steady improvement.

August 27, he was discharged relieved; by the last report on March 10, 1924, there was no subsequent trouble.

CASE 4.—E. H., a man, aged 28, entered hospital June 6, 1923, with the diagnosis of angina and mitral stenosis.

Family History.—His mother had high blood pressure at the age of 46, and his father had asthma.

Previous History.—No history of rheumatism or tonsillitis. He had pleurisy in 1917, otherwise his history was negative. The patient was married, and had three children.

Present Illness or Injury.—On May 5, at 7:30 a. m., after breakfast, the patient had pain in the region of the heart apex which got steadily worse, extending first to back of left side of neck, then across base of heart under the sternum where it stayed for one hour. The whole attack lasted for two hours. If he took a deep breath, the pain was worse and the heart beat rapidly. He had not worked since then by his physician's orders. The second attack was on the afternoon of May 8, at 3 p. m. There were two or three waves of pain, chiefly in the heart region. It was not in the shoulder or neck or substernally as before. The pain lasted off and on until 8:30 p. m. The patient took a good deal of medicine.

Teeth.—One crown but otherwise they were in good condition.

Chest.—No signs of a pathologic condition.

Heart.—The sound was very weak, systolic murmur loudest and roughest at apex, which was in the fifth space at the mammary line. The second pulmonic was accentuated.

May 11, at breakfast the patient had a weak spell and felt as though he would faint, and had to go out and sit down. He became dizzy but did not faint. The heart pounded but did not hurt. He had an ache in his back, in the lumbar region, which developed May 10.

May 12, he had a dull ache in the heart region and left arm the previous night from 4 p. m. until he went to sleep.

May 16, when the tube was passed to remove a test meal, the patient had an attack of angina, with pain radiating down the arm and down the outer side of the left leg. The arm pain was worse than the leg. He had pain May 15 in the back of the neck.

May 19, the patient had pain again on the previous night, not severe, but felt it in the left arm.

May 21, not much pain May 20, but two or three pains in apex region after going to bed.

May 22, pain in heart May 21, at night, caused by vomiting after a dose of castor oil. At 10 a. m. the patient was in operating room. The pulse was 60 and regular. Blood pressure, 120 systolic, 50 diastolic. Left superior cervical sympathetic ganglion removed.

May 23, the patient vomited in the morning but had no attack of pain associated with it, either in chest or arm. The left pupil was contracted and the left lid drooped slightly. He said he had once felt as if a bee was buzzing in his pocket over his heart, but had no pain whatever in the heart. There was irritation of the recurrent laryngeal, and the patient had trouble swallowing.

There was pain in the head over the left eye the previous night from 11 p. m. on, which he still had at noon. He swallowed better than on the previous day. He said his eye felt funny and half closed. It was sore to the touch or when he moved the eye. He had a slight conjunctivitis in that eye. Ether (?).

May 26, the patient had nightmare (dreamed he fell in a river) during the night and awoke with violent pounding of the heart, which he says shook the bed and caused him a panicky feeling but no pain. He swallowed without trouble. There was slight ptosis of the left eye lid. The left pupil was smaller than the right.

May 27, suddenly, yesterday afternoon, while lying quietly in bed, he had a buzzing feeling over an area the size of a dime just inside the left nipple. Two or three minutes later, he had a pain (dead aching in character) which differed from the former acute shooting pain. It was quite severe, extended to the top of the shoulder joint and down the inside of the left arm and down to the wrist. The fingers felt a tingling only, as if asleep. The pain lasted from five to ten minutes and gradually wore off. He moved about in bed, and a little later took his supper and later took soda and got rid of a good deal of gas. The pain, however, had gone. Later he described it as a gas pain. He was a good bit apprehensive and might have been frightened by the attack and exaggerated it, but he certainly had symptoms of an anginal attack.

May 31, the left conjunctivitis had about disappeared, but after reading it returned and he had pain in left supraorbital region. The contracted left pupil followed the left cervical sympathectomy. The patient was referred to the oculist, Dr. Blake, for recommendation.

June 1, the eye was examined by Dr. Blake, with the following report: The left pupil was smaller than the right. The reaction to light and in accommodation was normal Miosis was probably due to absence of tonic dilator impulses by way of cervical sympathetic. The fundus was normal in both eyes. Yesterday afternoon, while playing cards, he had a sudden feeling of oppression when breathing, his head was light and he had a choking sensation. He found he could breathe easily but felt the need of air. He felt faint and dizzy. He came back to the ward, his legs felt weak and his head felt as if he were floating on air. Took 2 drops of nitroglycerin and in thirty minutes was quite recovered. He lay down during this time. There was no immediate effect from the nitroglycerin. The nurse's record said immediate relief, but he had been getting better for from ten to fifteen minutes before the nitroglycerin. All functions of the body were being performed well, although a laxative at night had been used. Blood pressure was 110 systolic, 66 diastolic. The pulse was 60. The electrocardiograph showed only right, ventricular preponderance.

June 4, examination (P. K. B.) could not detect the slightest heart murmur. There was a reduplication of the first tone at the apex. The rate and rhythm were normal. There was nothing to indicate a valve lesion. Blood pressure, 110 systolic, 60 diastolic. Pulse, 68.

June 26, the patient still had a slight numbness in front of the left ear, and below it to just below the angle of the jaw. He was sore over the region of the lower parotid, and eating sour things caused pain. There was nothing found in the teeth, but both tonsils were big, cryptic and infected. He caught a cold which lasted for four or five days, and was pepless for a time. He was feeling well before that. Blood pressure, 106 systolic, 60 diastolic. Pulse 88. Tones of the heart semed absolutely normal. There was no change in size. Except for gas, he had no complaint, and when he had a great deal of gas, or overexerted, he had an ache with three or four successive heart beats, which lasted intermittently for half an hour. This continued until relieved of gas, and was worse when he was without food for longer than usual. Eating then relieved him, because he belched as soon as he had eaten.

CASE 5.—A. W. B., a man, aged 58, with a history of angina, was admitted to hospital on the service of Dr. Earl Greenwood, Aug. 3, 1923. The patient's chief complaint was pain in the precordium extending down the arm, lasting for the last nine or ten years.

Past History.—The patient was an inveterate smoker, a heavy drinker (not for the last four months) and a heavy eater. He had had measles and mumps at 17, with testicular involvement. About ten years ago, the patient had a severe attack of pain in the right upper quadrant, gallstones and no jaundice. He has had some urinary disturbance, with frequency at night, which has diminished lately since letting up on coffee; there was no burning on urination. Questionable primary syphilis, no secondaries.

Present Illness.—Nine or ten years ago the patient first noticed the onset of pain in the precordium following some exertion, of a dull aching type, periods of freedom occurring nearly every day. Attacks were brought on by cold air, anger, excitement or effort.

About one year ago, the patient undertook extra work involving nervous as well as physical strain. At this time the pain, of a sharp shooting type, became more severe, radiating down the left arm. The patient was not certain whether the pain originated in the precordium or the left arm, but both are associated. He stated that there was an area over the second rib painful to touch during attacks. There was no substernal pain. The patient has had five severe attacks during the last five days. During examination, he had a long attack lasting several hours, relieved after five doses of $\frac{1}{100}$ grain nitroglycerin hypodermically. He had been in the habit of taking large amounts of black coffee for the last year. He had been an inveterate smoker all his life. On the day of admission he used a considerable amount of alcohol in an effort to relieve the pain. He has had some shortness of breath lately but no swelling of the feet or ankles and no asthmatic attacks.

Physical Findings.—An elderly man, of florid complexion, and somewhat cyanotic was seen. The pupils were irregular and small (codein?). The teeth were in a very poor condition; the gums were discolored. The neck showed some pulsation in veins. The chest was thick, there was some dulness over the whole area and both lungs, especially at bases, elicited musical coarse rales; there was a small area of pleuritic rub in the right axilla posteriorly. The heart area of cardiac dulness was enlarged to the left 2 cm. outside the mid-clavicular line, the sounds were rapid and weak, with a faint systolic murmur at the apex. Blood pressure, 148 systolic, 100 diastolic.

Abdomen.—Protuberant. The liver was enlarged about one finger below the costal margin, somewhat tender, otherwise physical examination was negative. The Wassermann test was negative, and the urine was negative.

Roentgen-ray examination of the thorax showed "myocardial type of heart," some widening of the aorta, increased bronchial tree markings about the hilum, probably secondary to cardiac trouble.

August 9, the blood pressure was 138 systolic, 28 diastolic; August 10, 122 systolic, 80 diastolic; August 12, 118 systolic, 80 diastolic.

August 15, left cervical sympathectomy was performed. The anesthetic began at 12:25, the operation at 12:40 and ended at 2 p. m. The superior cervical ganglion was removed. The superior cardiac nerve and main trunk were resected. The pulse before operation was 88, after 110. Respiration, 19 before, after 28. Blood pressure just after operation (2 p. m.) was 90 systolic, 70 diastolic. At 8 p. m. it was 118 systolic, 78 diastolic.

August 16, the blood pressure was 112 systolic, 76 diastolic. The patient complained bitterly of pain in the left side of the neck and begged for relief. Nitroglycerin, $\frac{1}{50}$ grain hypodermically and $\frac{1}{50}$ grain by mouth, gave no relief, even repeating both doses. The pain he complained of seemed to be local, due to trauma of operation. He had no arm pain, which had been his worst symptom, till reminded of it at 8 p. m.

August 17, the blood pressure was 112 systolic, 70 diastolic; August 18, 98 systolic, 58 diastolic, sitting up; August 19, 118 systolic, 76 diastolic; August 20, 110 systolic, 72 diastolic; 128 systolic, 86 diastolic, lying down; August 21, 118 systolic, 74 diastolic, sitting up.

August 23, urine analysis, reaction, trace of albumin, specific gravity 1.015, occasional granular casts, many hyaline casts, no sugar.

Within the week after operation, he was up and walking to the sun porch. His chief complaint was air hunger. He had fair nights on phenolbarbital, 2 grains. His limbs began to swell within a few days of operation, the urine became less in spite of a diruetic every two hours. The pain was chiefly in the neck, but he complained at times each day of pain above the heart and in left forearm. A troublesome cough developed on the fifth day after operation, and the dyspnea grew worse. In spite of improvement in renal output, daily bowel movement, light diet and abundant stimulation, the heart from this point failed steadily and the dyspnea grew worse.

The patient died at 9 p. m., the tenth day after operation. During the last five days his one complaint was air hunger, and except for one attack of heart pain relieved by aromatic ammonia, there was no true angina. Necropsy was refused.

Clinical diagnosis: Advanced coronary disease with interstitial myocarditis. Death from failure of the heart.

CASE 6.—A. K., a man, aged 59, on Oct. 3, 1923, complained of upper substernal pain and flatulence off and on for the last three weeks. The pain came on after exertion and the attacks lasted for ten minutes, the patient having to sit and gasp for breath. Eight years ago he ran for a train and got out of breath, fell and could not speak for twenty minutes, so severe was the pain. The pain was located in the mid-chest across sternum, and generally shot from the elbow down to finger tips without affecting the shoulder or upper arm. No other radiation of pain was noted. Dyspnea accompanied the attacks. These come on an average of from six to seven times daily. There was no pain when in bed and quiet. There was a good deal of bloating and flatulence, which aggravated the chest pain. There was no dizziness. The disappearance of chest pain left him with a frontal headache. Cold sweat followed the attacks.

The pain began when he made the exertion eight years ago. He thought it was in the bronchial tube and throat, on account of the difficulty in getting his breath. Three years ago, in the Siskiyou Mountains, he had recurrence of the pains, and difficult breathing which became normal when the pain disappeared. The blood pressure was 118 systolic, 70 diastolic.

October 5, the patient had an eye examination. The external eyes were negative. The mediums were clear. The fundi were negative. Myelinized nerve fibers were present at the margin of the disks in both eyes.

Roentgen-ray examination of the chest, heart and aorta showed considerable hypertrophy of the left ventricle. The aorta was increased in density. The transverse portion of the arch measured 7.5 cm., which is a normal measurement. The heart measured 15 cm. in the transverse diameter, 17 cm. in the diagonal diameter. Conclusion: Aside from the unusual density of the aorta, no pathologic findings were seen.

October 6, the patient had no attacks of pain on October 4, but had two attacks October 5, one at 4 p. m., lasting from five to ten minutes; one at 8 p. m., lasting from three to four minutes. At 4 a. m. he was awakened by an attack more severe than usual and lasting for ten minutes. He took no medicine, and perspired freely after the attack. He had no special trouble with breathing, but in this attack he felt that he must draw in a long breath. The pain was always located below the upper sternum and ran over toward the upper axilla. Sometimes the pain shot down the forearms, and that morning especially he felt it in the right forearm; it semed to terminate in the thenar eminence. It did not radiate to the neck, but did at times radiate to the left shoulder, never to the right. Never in the back of the head, but when the pain (substernal) had lessened he had a frontal headache for from twenty to thirty minutes. The vision was never disturbed. The patient has had "touches of pain before in the right forearm." Nitroglycerin, $\frac{1}{100}$ grain, was prescribed for attacks. The blood pressure was 138 systolic, 98 diastolic.

October 8, at 8 p. m. the patient had an acute attack of pain in the substernal region, radiating to the back between the scapulae to both arms. It was not relieved by two doses of nitroglycerin at one-half hour intervals. There was slight relief from amyl nitrite.

October 9, he had bad anginal attacks during the night, and pain off and on all night. He was given nitroglycerin, amyl nitrite and morphin. He vomited the food this morning that he had eaten the previous afternoon. He had eaten marshmallows before supper.

October 11, roentgenogram of the chest showed opaque shadow at the left base, probably due to slightly thickened pleura. The stomach filled in the usual manner, and was normal in outline, size and position. No filling defects were seen. Roentgenograms of the gallbladder showed a rounded shadow under the liver. The stomach was empty in the usual six hours time. A twenty-four hour examination showed the colon well outlined.

The findings were marked spasm of the pylorus and bulb, in conjunction with the round shadow under the liver pointed to a condition of chronic cholecystitis.

October 13, the patient had no pain since October 8.

October 14, he had an attack the previous night, relieved by nitroglycerin.

Family History.—Negative.

Habits.—The patient did not smoke or drink.

Past History.—Hemorrhoidectomy in 1916. Otherwise negative.

Examination.—A well developed, middle-aged man, lying quietly in bed, in no apparent pain was seen. The head, eyes and nose were normal. The mouth had plates. The tonsils were negative. The chest was symmetrical, with normal expansion. The lungs were resonant throughout; there were no adventitious sounds. The heart apex beat was best heard in the fifth intercostal space nipple line. The sounds were distant and regular; there was no murmur. The abdomen was negative. The extremities were negative.

November 12, the patient returned from leave of absence (thirty days). The usual symptoms were present. Some days he would have from three to four anginal attacks, others were free from pain. The slightest exertion brought on an attack of precordial pain radiating down the left arm. There was shortness of breath during the attack and the patient could not breathe. Also cold sweat appeared. The patient had a slight postcervical ache. He had not become any better or any worse.

November 13, on ascending an incline yesterday, he had to stop twice on account of heart pain. Vital capacity 2,800 c.c. The blood pressure was 100 systolic, 72 diastolic.

November 14, the electrocardiograph was made yesterday. The patient had no attacks. For the last month he has had no day without attacks, from two to six a day, depending on exertion. These attacks were relieved with nitroglycerin. Tincture of digitalis, 20 drops three times daily was prescribed.

November 15, there was an abrasion on the right side of the epiglottis but no evidence of any bone in the larynx or upper trachea.

November 17, the blood pressure was 116 systolic, 80 diastolic.

November 19, the left superior cervical ganglion was removed, under local anesthesia, by Dr. Coffey.

November 20, the operation produced no reaction in the patient. After the wound was closed, the blood pressure was 118 systolic, 78 diastolic, and the pulse 68, regular and full.

During the operation the patient experienced a pressure pain above the midclavicular region over to side of larynx. When the superior ganglion was grasped with forceps, he felt the same pressure pain at the base of the brain. (Distinctly not the burning pain as described by H., the other patient done under local anesthesia.) He had blurred vision in the left eye in the morning.

He had no numbness at angle of left jaw, which all the other patients have described. Early on the afternoon of November 19, the patient had a pain in the left breast region lasting for ten minutes, unlike any pain he had had before. He thought it was in his heart. It did not radiate to the neck or arm. The patient stated that he was very glad he had the operation done under local anesthesia, that the pain at no time was disturbing and was only momentary. He said he would be willing to go through it again the next day if necessary.

November 21, the patient had been feeling well all day until 4 p. m. when he developed slight pain down the left forearm. The pain lasted for one hour, and was just like attacks in the arm before operation. There was no substernal or precordial pain. There was slight numbness over the left angle of the jaw, otherwise he was feeling all right. There was a slight bowel movement, as cascara had been given.

November 23, the patient was up, November 22, for three or four hours. He had slept well the previous night until 3 a. m. He awoke with pain in the region of the apex of the heart, which extended all over the precordial region along the wound and up to the left temple region. He felt it in the left eye also, and down the whole left arm, especially in left forearm flexor surface as far as wrist. The pain lasted for thirty minutes. He was given $\frac{1}{150}$ grain of nitroglycerin hypodermically, with relief in ten minutes. The patient stated his evening meal was the ordinary diet. He felt no discomfort following, and slept until 3 p. m., when he was awakened by a pain in the epigastrium (as far as the umbilicus) which extended up the sternum to the neck. It was unlike any pain he had experienced during his previous anginal attacks. However, he stated there was an associated pain over the heart extending up to the neck and eye (left), to the wound and down the left arm and forearm, similar to the pervious anginal attack. The pain was quite severe, and he felt like vomiting during the attack. The patient thinks this attack was due to indigestion. He had been given cascara and mineral oil the night before, and this was followed by some epigastric distress. In addition to this, he had a decided impression of fear, due to the fact that a week before an anginal patient had died after being six hours in the ward in an attack, and the afternoon before, another heart patient came in, in a state of collapse, after being allowed to dress and take a short walk. The patient went to bed at once, saying he didn't want anything like that to happen to him. These facts are recorded, together with a notation of digestive disturbance, for what value they may have in connection with the nervous origin of attack.

November 24, at 6:35 p. m., the patient suddenly was seized with sharp pain about the sternum, also beneath the lower end of the body of the sternum, also pain over the heart, which did not radiate to the neck or shoulder, but was quite agonizing from the elbow down. The patient also complained of pain about the mastoid, a sensation similar to that of earache. This entire attack lasted only for five minutes after taking 2 drops of nitroglycerin. Profuse sweating of the lower extremities followed. The blood pressure was 128 systolic, 90 diastolic (Tycos) during the attack. The patient described the attack as "light" compared to some he had had. The left pupil was contracted and the left upper lid drooped slightly.

A three weeks' leave of absence was given the patient as he had a marked suggestibility and it was quite impossible to evaluate his symptoms.

December 19, the patient returned complaining of (1) a dull aching over a small spot in the center of the left shoulder toward the back; (2) pain at the angle of left side of jaw on beginning to chew, for three or four movements of the jaw. This began about December 1, and continued since. The patient did not notice anything but numbness at other times; (3) pain and tenderness over a small area over the left anterior occipital region about the level of top of ear; (4) on December 7, 8 and 16, he had pain around a small area over the heart apex, which worked back under the shoulder blade instead of to the front as

formerly. He took nitroglycerin once with relief. Again, on December 18, when returning to San Francisco, the patient, while carrying a bag, hurried, and had another attack. Exertion had been a factor in each attack.

December 22, the blood pressure was 118 systolic, 76 diastolic.

December 24, at the eye examination it was found the right pupil was larger than the left, both reacted to light and in accommodation. The left eye slit was narrower than the right. The muscle movements of both eyes were good.

December 26, on the night of December 23, at 7 p. m., the patient had pain in the neighborhood of the left nipple over an area of 10 cm. across. He had a cramp in the flexor surface (lower third) of the left forearm, which was relieved by nitroglycerin. The cardiac pain did not radiate. The patient did nothing unusual except eat a hearty supper at 5 p. m. He was in bed when the pain began. He was taking pyramidon 2½ grains for pain below the left ear, anterior to operation wound and extending up in front of ear. The ear pained only when he began to eat. The neuralgic pain up behind the ear did not recur after he took the two doses of pyramidon, grains 2½, at 8 a. m. and 5 p. m.

December 27, at 7:35 p. m., the blood pressure was 122 systolic, 86 diastolic. The patient stated he was suddenly seized with severe aching pains over the precordium, also cramp-like pain in the left wrist, and extending about 5 inches (12.7 cm.) up the left forearm. There was no radiation of pain to neck, shoulder, or to mastoid process. There was no substernal pain. Nitroglycerin, 1/100 grain. The attack lasted about three minutes.

December 29, the patient had three attacks December 28. The first was while wandering around town going up a hill; he had pain only in apex of the heart and none in the arm; second one while walking, not hurrying; third one while in bed at 7:30 p. m., two hours after eating.

Jan. 2, 1924, the patient had no attacks. He complained of pain just in front of the lobe of the left ear and below the angle of jaw. It was tender also to pressure, and worse when he began to eat. At times he had it without eating.

Case 7.—J. B., a man, aged 59, a farmer by occupation, was referred by Dr. O. F. Johnson, Dec. 17, 1923, on the complaint of pain in the chest. Three or four years ago, when walking one evening, he had occasional attacks of pain which began in the shoulder and extended down through the front part of the left arm to just below the wrist. He did not feel it in the neck or head. He felt it very slightly under the sternum at that time. For three years, the attacks continued like this, with the chief pain in the arm. They occurred only on exertion and from one to four times a month, and would pass off in a few minutes when he rested. There was some dyspnea accompanying the attacks. The patient never had any symptoms on the right side.

About one year ago, without any known cause, he began to have some severe pain substernally, occurring more frequently and lasting longer, never radiating to the neck or back of head.

On admission, he felt the pain first across the lower sternum below the nipples and extending out nearly to them, chiefly in the center below the sternum. The pain sometimes seemed to extend out beyond the nipples and sometimes a ½ inch (1.27 cm.) below them. He never felt it in the back except in the upper posterior part of the shoulder, and down the flexor surface of the arm to a point just below the wrist. Sometimes it came without any exertion. It seemed worse in cold weather.

Examination.—An obese Italian was seen whose blood pressure was 200 systolic, 118 diastolic.

Heart rhythm was normal, the rate 60. The first tone was not very plain anyhere, the second was much exaggerated and ringing over the aorta, but there were no murmurs.

Lungs: There were no râles in the base of the lungs.

The upper teeth were on a plate. There were abscesses around the roots of the lower teeth.

The tonsils were not unusual.

The pupils were equal and reacted to light. The background was not visible because of marked contraction. The knee jerks were equal. He had had nycturia for the last six or seven years. Roentgenogram disclosed a shoe-shaped heart, marked hypertrophy and dilatation and a broadened aorta.

The Wassermann test was negative.

Electrocardiogram: Marked left ventricular preponderance, negative T wave in Lead I; vital capacity 3,100 or 83 per cent.

The urine was negative.

Operation under local anesthesia was done by Dr. Coffey, who removed the left superior ganglion. The patient smoked daily after the operation, and was discharged in eight days. He had a mild attack of appendicitis in this interval.

CASE 8.—H. J. H., a man, aged 68, a purchasing agent, was seen Jan. 16, 1924, with an admission diagnosis of angina pectoris.

Family History.—Negative.

Past History.—He denied having syphilis or any serious illnesses.

Present Illness.—The patient was told in 1910 that he had arteriosclerosis. He complained of headaches, vertigo and pain in sides of neck toward the back. This neck pain recurred in attacks for fully eight years before he began to have attacks of pain substernally, which radiated down the left arm. These attacks lasted only for a moment and were relieved by spirits of nitroglycerin, from one-half to 2 drops. The attacks were not very frequent, occurring about once a month, and according to the patient seemed to be brought on by worry only.

Since 1920, the attacks lasted longer, from about four to five minutes, and came on after any exertion such as walking from four to five blocks vigorously.

In 1922, the patient had pain in the base of the brain, and vertigo with the anginal attacks. The attacks always began with pain under the sternum, radiating across the chest and down the outside of the arm, only very rarely down inside of arm, sometimes entering the shoulder. The pain always went to back about to the level of the fifth dorsal in middle line. Occasionally, there was a slight pain in the back of the neck, which was not very severe and had not the same acute character. The patient did not smoke, and very rarely drank. Cold air did not provoke attacks. He might go for a few days without attacks, then have from one to four a day. For at least two months he has had two or more attacks a day, when he did have them.

The Wassermann test was negative.

The electrocardiagram showed slight ventrical preponderance.

Vital capacity, 2,550 c.c., 68 per cent.

Screen Examination.—The heart was normal in outline, size and position. The aorta was considerably thickened, and measured 8.5 cm. in the transverse diameter, anteroposterior view. The heart measured 15 cm. in the longitudinal diameter and 12 cm. in the transverse diameter.

Roentgenograms, taken at 2 meters, showed the diagonal diameter to have been 15 cm. and the transverse 12.5 cm. The transverse diameter of the aorta was 6 cm. The antero-oblique position showed a marked thickening of the descending aorta; this practically filled the posterior mediastinum.

Conclusion.—Chronic aortitis of the thoracic aorta. Urine, negative.

During a week's rest in bed, patient had no severe pain and only a few slight pains.

January 23, notes made during operation, there was a systolic heart murmur with slight enlargement of the heart and broadening of the aorta. Local anesthetic was begun at 10:10 a. m. The patient was under considerable nervous tension, and in a cold sweat. At 10:35 he began having a slight anginal substernal pain. At 10:40 the pulse was regular, 120. Blood pressure, 220 systolic, 128 diastolic. One hour before operation the blood pressure was 178 systolic, 116 diastolic.

At 10:41 as the anginal pain still continued 1/75 grain nitroglycerin, 3 drops of a 1 per cent. solution was given on the tongue. The pain disappeared in three minutes. The blood pressure fell so that the systolic could not be taken, diastolic was about 110.

11:10: After section of trunk and superior cardiac branch the blood pressure was 178 systolic, 100 diastolic. Pulse, 108. Contracted pupil.

11:15: Blood pressure, 165 systolic, 88 diastolic.

11:25: Pulse, 108.

11:35: Blood pressure, 162 systolic, 90 diastolic.

Pain in the middle of the left shoulder toward the back was felt when the branches of the cervical plexus were touched. This (Dr. Coffey says) had nothing to do with the sympathetic system. In this operation, the cervical plexus was exposed for the first time.

January 24, the patient felt fine. One extra systole was noted. The blood pressure was 190 systolic, 120 diastolic. He complained only of a few short attacks of hiccough. His stomach was much upset January 23, and had not quite recovered. The breath was foul.

January 25, the patient had four attacks of hiccough in the night. He had no bowel movement the previous day. With the hiccough he had pain, each time he hiccoughed, from the midsternal region to the back. (Injury to phrenic or pneumogastric?). Compound spirit of ether was prescribed.

January 30, the patient had three attacks of hiccough on the nights of January 25 and 26, two the next night, one the night after and none since. The blood pressure was 146 systolic, 88 diastolic.

Feb. 1, 1924. After walking about 400 yards yesterday, the patient lay down for a while and had a number of visitors, and with no known exciting cause had a moderately severe anginal attack at 6 p. m., quite like those he had already experienced, upper substernal pain going through to back between shoulders and slightly down the left arm. He took no medicine. The pain lasted for ten minutes and was not bad enough to cause him to think of medicine. The blood pressure was 172 systolic, 86 diastolic.

February 2, he had no subsequent attacks. His weight was 139½ pounds (63 kg.) before operation, in wrapper, 152 pounds (68.9 kg.) dressed. His tongue was clean.

February 22, Dr. Harry Weil reported the blood pressure was 129 systolic, 88 diastolic. The patient has had no attack of pain. He walked a block or two at a time and ate carefully. His feet became cold, but not more so than before. He had some swelling in the site of operation. The ear and shaved part of the face were numb. The left eye was inflamed, the lid drooped slightly, the left pupil was smaller than the right, though both reacted promptly to light. Every time he chewed on the left side, there was a single sharp severe pain felt in the molars in the upper left jaw. The second bite was free from pain. He might get it without chewing, and thought it might be caused by his teeth. But the regions in front of and behind the lobe of left ear and in the shoulder point were tender where Cases 1, 3 and 6 were tender. When seen May 15, he reported no further attacks. Exercises moderately.

CASES IN WHICH OUR METHOD WAS USED BY OTHERS

CASE 9.—W. H., reported previously,[2] was the first patient operated on under local anesthesia, and the extent of the operation was severing the superior cardiac nerve and the main trunk of the left cervical sympathetic below the superior ganglion.

Following the operation in the early spring of 1922, the patient returned to his work as a bridge carpenter and continued well until November, 1923, when he was transferred to a job in the mountains at an elevation of 5,900 feet. He caught a severe cold, and in a few days developed a severe and sudden pain over the heart apex and in the upper left central epigastrium just under the ribs. The pain radiated to the left shoulder and elbow but not to the neck, nor

was it substernal in this attack. The attack was mild, but the pain continued for nine days with four or five intermissions a day. He was short of breath and had a disturbing cough, and night sweats on three occasions in the next ten days before entering the hospital. The pain differed from previous pain in not appearing in the neck, and in being especially severe in the region of the heart apex and in extending straight through into the back.

Examination revealed his heart was fibrillating. Blood pressure was 90 systolic, 70 diastolic. Wrist pulse, 144. There were râles in both apexes (activation of old tuberculosis). On digitalis, quinidin and strychnin the fibrillation ceased on the tenth day after entering the hospital. In this period, the patient had repeated attacks of angina always with the same distribution of the pain. The heart was found to be fibrillating in all the attacks that followed in the next five weeks, although the fibrillation was paroxysmal and of short duration. Fibrillation was not always present in attacks of pain. The patient remained continuously on doses of 2 grains of quinidin and 1/30 grain of strychnin three times daily. For the last ten days of his stay in the hospital, he complained of a severe burning feeling in the region of the heart apex. These were relieved promptly by nitroglycerin. The electrocardiogram showed the inverted T in Lead III, which was not present when the patient was first operated on in January, 1922.

Consideration was given to the operation on this patient suggested by Daniélopolu and Hristide[13] and a complete study was made of the extent of the surgery necessary in this case and in Case 6. These authors suggest suppressing the principal sensory paths of the heart and aorta through resection of the posterior roots of the eighth cervical to the fourth dorsal. They report their researches on cardiac sensibility and the possibility of ameliorating angina by resection of the posterior fibers of the spinal nerves, beginning the article by the statement that "we know positively that the radiation of the anginal pain is due to irritation of the centripetal paths, which contain both the sensory cardiac fibers and those to the cutaneous areas corresponding." They then raised the question of whether the painful syndrome in angina is visceral and cutaneous, or only cutaneous, as claimed by Mackenzie, thus marking the referred pains in the arm and neck as reflex in the cerebrospinal fibers of the medullary segments corresponding to the area of the heart or vessels affected. Other authors are of the opinion that the pain is provoked by a visceral disease, and is felt at the same time in the viscera and in the corresponding cutaneous area. The researches of Daniélopolu and Hristide favor the hypothesis of Mackenzie that the pain is transmitted, that other sensations, palpitation, etc., are felt directly in the viscera.

Their observations were made on a syphilitic patient, aged 41, with aortic insufficiency and angina, which began July, 1918, following an emotional disturbance, when he had hard pain for a few moments. Attacks occurred rarely at first but later more frequently. Eight months after the first attack, one occurred at night, and following this, from ten to twenty a day occurred following the least effort or emotion. The pain was controlled with nitrite of amyl. Any food would produce it and the attacks came many times at night. The pain was retrosternal, and occurred also in the anterior surface of the left half of the thorax, radiating down the arm to the elbow. The arm pains were relieved somewhat by friction on the skin of the arm provoking strong irritation. The heart rhythm was regular, the rate from 74 to 94. There was some dilation of the right heart. The Wassermann test was positive. There was nothing special in the urine.

Anesthesia of the left dorsal spinal nerves in the intercostal tract and of the ramicommunicantes was done with 8 cg. of procain. The second and third dorsal branches were anestethized, because minute study led them to localize

13. Daniélopolu, D., and Hristide: Bull. et mém. Soc. méd. d. hôp. de Par. 47:69 (Jan. 25) 1923.

the pain in the cutaneous areas supplied by these branches. Half an hour after, the patient was put through numerous tests, with a ten minute rest between, and although the heart rate mounted from 84 to 140 and the patient suffered from great shortness of breath and palpitation, there was no pain. The same efforts were repeated at the end of six hours when the effect of the procain had partly passed off. Characteristic pain followed, but nothing so severe as before the anesthesia, and after twenty-four hours, the least effort provoked a violent attack of pain. The authors drew the deductions as a result: (1) that anginal pain is exclusively a transmitted pain; and is conveyed to the cutaneous areas of the same medullary segments that supply the heart and aorta, and (2) that all the sensory manifestations coming from the heart are not transmitted. In spite of the anesthesia of the corresponding spinal nerves, the patient was conscious of palpitations. If we except the anguish which appears to be due in part to the contraction of the pectoral and intercostal muscles, the other sensory manifestations of the heart, such as palpitation, sensations of extra systoles and those provoked by the onset of an attack of paroxysmal tachycardia, all have their origin in the affected viscera.

The authors claim for their method that they rob anginal attacks of the pain by suppressing the sensibility of the spinal nerves. These authors draw the further deduction that the Jonnesco operation, proposed by François Franck, of extirpation of the cervical-thoracic sympathetic system and successfully carried out twice by Jonnesco, the second time bilaterally, is inadmissible because it takes away from the heart nearly all of the extracardiac motor fibers except the sympathetic fibers of the vagus, and lessens the muscular function of the heart already diseased by angina. They claim further that this constitutes a predisposition to a systole of the left heart, contributing to pulmonary edema, a grave complication, which they state is frequently met with in angina with hypertension; and still further that this extirpation of the vasomotor fibers of the sympathetic system of the coronary arteries, which are probably vasodilators, diminishes coronary flow already jeopardized in angina. They claim that the resection of the posterior roots of the eighth cervical, first, second, third and fourth dorsal nerves, is a much less serious operation and does not touch the motor nerves of the heart. They admit the criticism that their operation does not suppress all of the centripetal paths in the cervical sympathetic cord, and state that if it is necessary they can be cut, but not resected. Finally they claim that the Jonnesco operation does not completely suppress all the cardio-aortic sensory tracts because the vagus is left intact.

In one case which they report, which came to operation, they cut only the second dorsal nerve, because the patient had a violent anginal attack during the operation.

Further communications are promised after prolonged observation of interesting phenomena, following their incomplete operation.

There seemed too much uncertainty about this operation and it is anything but simple. Besides, as Ranson points out, all the spinal connections would have to be cut very certainly, and possibly on both sides to insure freedom from pain.

From November, 1923, to the time this paper was written, the patient had spent most of the time in the hospital, under observation. For nearly two months he was in bed. Two grains quinidin and $\frac{1}{30}$ strychnin had influence in preventing his heart from fibrillating. A curious thing was made very evident on numerous occasions, and that was that his anginal attacks were followed immediately by fibrillation.

In addition to symptoms already described, he had attacks of apparent pain in the apex, which he felt as a drawing pain under the angle of the scapula on that same side. Occasionally these attacks were so severe that it took three doses of $\frac{1}{100}$ grain of nitroglycerin to relieve them. On one occasion the attack lasted one-half hour without any relief. In the two months in bed before the second operation in March, 1924, he had twelve attacks.

The second operation was done under local anesthesia, and the superior ganglion was removed, and from that time to April 26, he had no attacks what-

ever although he was up and about all the time and took a moderate amount of exercise. Since the operation, however, he had a cough and some difficulty in swallowing. He did not swallow at all for twenty-four hours after the operation, and for a week thereafter he had a little trouble with swallowing liquids. He had tenderness in the back part of the left shoulder which disappeared in a few days. There was a slight pain in the angle of the left jaw when he began to eat at meals. Fibrillation occurred on several occasions since the operation, once lasting for three hours, but without pain. After the second operation, there was a roaring in the left ear and apparent deafness. The Rennie test was positive in the left ear. Bone conduction was normal. There was no disturbance of equilibrium. The throat specialist stated that the pain he complained of in the larynx after the removal of the stitches in the neck, was associated with a vesicular eruption on the larynx looking like herpes. This did not come for ten days after the operation.

Case 10 (Personal communication from Dr. Howard Naffsinger).—P. L., a man, aged 63, whose occupation was gardener and farmer. The diagnosis on entrance was angina pectoris and diabetes.

Past History.—Patient lived in France from birth until aged 7 years, New York and vicinity from age of 27 to 45, California from age of 45 to 63. He had measles at 24, a mild attack with no sequelae. He had a mild attack of influenza at 30. He denies venereal history.

Present Illness.—Twenty years ago the patient began having attacks of precordial pain. These came after exertion and especially followed exercise after meals. At first they would be from one to six months apart, but gradually became more frequent. For a period of six months they came on at the slightest exertion, so he had to stop work entirely for a period. The attacks lasted from three to fifteen minutes. He had to stop whatever he was doing. The pain was located just to the left of the lower part of the sternum, and he felt as though he were being torn apart. When the attack was severe, the patient did not fear death, but did feel a sense of impending death. In recent years, a feeling of numbness extended from the anterior part of the shoulder down the left arm to the wrist accompanying the attacks. Nitroglycerin, prescribed in the outpatient department, helped to cure the attacks. There were no symptoms of diabetes, but the urine was found to contain sugar, 2 plus. The blood chemistry sugar was 165. The patient was put on a diet, and the blood and urine became negative for sugar.

Examination.—Irregular pulse. There was deep tenderness on the left side of the neck and shoulder. The chest was emphysematous and barrel shaped. There was an area of tenderness to percussion in the fourth left interspace just within the nipple line. The lungs were essentially normal except for hypostatic râles at both apexes. The heart was of normal size, and the sounds were of good quality. The second auricular was greater than the second pulmonic. A soft systolic murmur was heard at the apex only. Blood pressure varied from 205 systolic, 88 diastolic, to 160 systolic, 60 diastolic. There were palpable epitrochleas in both arms. The reflexes were all normal.

Laboratory Examination.—Electrocardiogram showed the left axis deviation of plus 20, inverted T2 and T3. Auriculoventricular interval was at four twenty-fifths of a second. The urine and the blood were both normal. The Wassermann test was negative.

Course in Hospital.—The patient had attacks of pain after walking from one to three times the length of the ward. On January 11, under local anesthesia, Dr. Naffsinger removed the superior cervical ganglion on the left. At one time during the operation the patient felt pain under the left clavicle similar to the pain he had been having, but not so severe.

Postoperative Course.—The day after operation, the blood pressure varied from 124 systolic, 90 diastolic to 164 systolic, 60 diastolic. There was endophthalmitis with a small pupil that reacted to light on the left. There was no

hyperesthesia or pain. The patient was able to walk the length of the ward twenty times without pain. He continued to be in good condition and unaffected by exercise. However, on the third day postoperatively, the patient developed a dull epigastric and substernal pain, not definitely related to exercise, not so severe as the previous pain and with no sensation of tearing or pulling.

January 17, the patient was discharged. His condition was much improved. The heart findings were similar to those on entry except that there was no systolic murmur and the blood pressure was 175 systolic, 80 diastolic.

The diagnosis was generalized arteriosclerosis with chronic interstitial myocarditis and angina pectoris.

On January 25, the following note was made in the outpatient record: The day after operation the patient was able to walk a considerable distance without pain. He could then walk about ten blocks at an ordinary gait without symptoms. He might then get a gripping sensation referred to the heart region, which is different from the former heart pain. There was no dyspnea. The blood pressure was 200 systolic, 98 diastolic.

February 8, the patient had been at work since the last visit, tending chickens, pruning vines, etc. He did not work all day, but as much as he could. He had been feeling well until the last three days, since then he noticed some pounding of the heart and described a somewhat painful sensation over it. His pulse in the morning showed several extra systoles a minute, and from his description it was evidently the occurrence of these that he felt. Blood pressure was 196 systolic, 100 diastolic, with the first beat after the extra systoles were over 200.

REVIEW OF CASES REPORTED BY OTHERS, AFTER LEFT CERVICAL SYMPATHECTOMY

CASE 11.—Dr. M. A. Mortensen, of Battle Creek Sanatorium, Michigan, wrote us of a patient on whom he performed a left cervical sympathectomy. Unfortunately about two weeks afterward the patient died, but not of angina. The fact that the patient was a morphin fiend is set down as contributing to the death.

"It is, however, an interesting point to me that after the sympathectomy he did not have the typical angina pains. He had pains in the left shoulder which extended as low as the clavicle and which were due to the surgical trauma. Relief of the pain by the use of nitroglycerin failed entirely, and the patient stated that this was not the same pain he had formerly had. Furthermore, he stopped taking nitroglycerin after the second day, while before the operation he was taking it from thirty to forty times in the twenty-four hours, in doses of one-fiftieth to one-hundredth grain."

CASE 12.—Dr. Alexander Lambert, the correspondent, gave the following facts in regard to a patient operated on by Adrian Lambert in the fall of 1923. The patient was a woman, age not stated.

"An extremely poor subject for surgery, because she was so nervous, overwrought and physically exhausted and very nearly at the end of her rope, after two years of practically a *status anginosis.*

"The left superior ganglion was removed.

"She had a stormy month's convalescence, in which the irritation of the spinal accessory caused a terribly painful spasm in the trapezius muscles. Also taking out the superior sympathetic ganglion made a very painful set of reflexes along the ear and side of the neck."

The final result, however, "was to relieve entirely the pain on exertion and to enable the patient to go upstairs comfortably like an ordinary person, which she had not done for two years; to be able to sleep on either side, a new experience for her; and the cessation of intense pounding in her heart and rapidity of action on slight exertion."

Dr. H. M. Marvin of the Yale Medical School, in a personal communication, reports the following two cases with a very definite contribution to the blood pressure studies during operation. His two patients were operated on by Dr. S. C. Harvey.

CASE 13.—The patient was a married Englishwoman, aged 54, who had been the subject of a few attacks of angina, classical in character, location and radiation, several years previously. They were followed by a period of freedom from pain, which was abruptly terminated by a seizure interpreted by us as due to coronary closure; intense substernal pain lasting for three days, relieved only partially by large doses of morphin and followed by intense weakness and dyspnea. Within the ensuing five weeks, even at rest in bed, she had four severe anginal attacks; shortly after the fourth, she came to the hospital.

Her past history was negative, except for typhoid fever and pneumonia in early adult life. She had scarlet fever in childhood, and frequent sore throats until a few years ago.

Family History.—The patient's first husband died of pulmonary tuberculosis. By him she had one miscarriage at six months, followed by a normal child who still lives. She had no children by her second husband. Of three sisters, two are said to have died of heart trouble.

Physical Examination.—Entirely negative except for evidences of heart failure; extreme dyspnea, orthopnea and weakness. The heart was not enlarged, the rhythm was regular, the sounds somewhat faint and no murmurs. The lungs were clear. The abdomen was normal. There was no edema of the extremities. Electrocardiogram was normal. Six-foot plate of the heart showed normal outline and no enlargement. Urine, blood and stools showed no abnormalities. Vital capacity was 42 per cent. of normal.

The operation of the left cervical sympathectomy was performed by Dr. Harvey, who evulsed the superior ganglion. The operation was on April 18, and since April 22 the patient suffered severely with pain and extreme hyperesthesia over the left side of the face, scalp, neck, shoulder, mammary and scapular areas, and frequently extending down the arm and forearm into the hand. Her symptoms resembled closely those attributed by Sluder to disturbance of the sphenopalatine ganglion. In the six months since the pain disappeared, it has been constant with the exception of three days, when it completely disappeared, only to recur as before at the end of that time.

It is interesting to note also that, although the operation relieved her completely of the precordial pain, it did not apparently stop the attacks. Four times since the operation, she has had attacks characterized by profound weakness, generalized sweating, fear of impending death, and a "queer, all-gone feeling" in the chest, symptoms which previously accompanied the anginal seizures; but there has been no actual pain with these later ones.

Our impression was that the patient had coronary closure six weeks prior to admission, in addition to typical angina. Operation was performed on April 18, 1923. The anesthesia was 0.5 per cent. solution of procain. The cervical sympathetic was severed (left side), and the superior ganglion extirpated.

During the year that elapsed, the patient was in almost constant misery. With the exception of a few days when in the hospital, she had increasing pain involving the left half of face, scalp, neck, shoulder, left breast, and part of left upper arm. Within the last few months, signs of congestive heart failure (cyanosis, edema, etc.) have begun to appear. She has been many times under observation; the typical angina seizures, similar in all respects to those before operation, have been noted. There were typical eye and pupillary changes following the operation.

CASE 14.—A man, aged 48, an Italian, had syphilitic aortitis, aorta insufficiency, and anginal attacks provoked by any moderate exertion or excitement. He had been having attacks for four years, which were growing worse within the last six months. The symptoms were always aggravated by antisyphilitic therapy. There were no signs or symptoms of congestive heart failure; the heart was not enlarged to physical examination or as shown in a six-foot roentgen-ray plate.

The operation was similar to that in Case 13, but with the addition of some gas-oxygen several times during the procedure, as the local anesthesia was not

entirely effective in abolishing pain. The left cervical sympathetic fibers were resected, with evulsion of the superior ganglion. Pain in the left side of the face and neck appeared on the fourth day, but disappeared within about ten days. The course of the patient was followed for eight weeks, during which time he had no recurrence of the chest pain, and found that he could do whatever he wished in the way of exertion. He considered himself cured, and returned to Italy, whence we have been unable to trace him.

The blood pressure did not show any significant change either during or after the operation. I took the pressures every two minutes throughout the operation, and three times a day for a week afterward. In both cases, it was a little higher at the conclusion of the procedure than at the beginning, but not as much as 10 mm.

Electrodiograms were also taken throughout the operation at intervals of several minutes, and at the moment of cutting the nerves and evulsing the ganglions. The T waves were upright in all leads of both patients, and no changes of any sort were found. Tracings taken several weeks later remained unchanged.

CASE 15.—Holmes [14] reported before the Chicago Society of Internal Medicine, Feb. 25, 1924, a case of angina successfully operated on by Dr. Wm. Shackleton.

The patient was a woman, aged 53, white, who was admitted to Wesley Memorial Hospital on Jan. 29, 1923, for treatment of cardiac failure. For about one year prior to the appearance of edema, dyspnea, and other signs of decompensation, she had vague pains in the precordium and left arm. During the first part of her hospital residence she did not complain of pain. The first attack of typical angina followed the effort of getting into a wheel chair. Thereafter, pain recurred with increasing frequency and severity until the slightest exertion was sufficient to bring it on. Nitroglycerin and amyl nitrite gave almost immediate relief but the subsequent prostration was extreme. On July 12, 1923, the superior cervical sympathetic ganglion was removed by Dr. Wm. E. Shackleton and saved for histologic study. The patient was discharged from the hospital Aug. 6, 1923, and soon thereafter was earning her living as a nurse in charge of two small children. At no time following the operation did she experience pain either in the thorax or in the left arm. Just a few days before the Christmas holidays she started on an automobile trip to Atlanta, Ga. En route she became ill and was admitted to the Nashville General Hospital. A letter from the hospital, dated Dec. 29, 1923, stated that she complained of severe abdominal pain, nausea, vomiting and of precordial pain just before her admission. The temperature, pulse, respiration, blood pressure, etc., were reported to be about normal. She recovered from this undiagnosed illness, and we understand she was discharged from the hospital, but her subsequent history is unknown. During the six and one-half months she remained under our observation, she did not have a single recurrence either of thoracic or brachial pain.

Holmes had the advantage of the stimulating and helpful interest of Ranson,[15] to whom we owe a grateful acknowledgment of his similar help to us. Ranson made it clear to us that no sensory fibers from the heart were to be found above the middle cervical ganglion, and the relaying of pain could only be stopped by the removal of the lower cervical and first thoracic ganglions. The studies of Holmes and Ranson are valuable contributions to the understanding of angina, but we are at a loss to explain the burning sensation in the base of the brain of one case, when the superior cervical ganglion was stimulated by a weak galvanic current during operation, and after the superior cardiac nerve and the main trunk had been severed. Holmes quotes Langley as finding no evidence of

14. W. H. Holmes of the Department of Medicine, Northwestern University, Chicago.

15. S. W. Ranson of the Department of Anatomy of the Northwestern University, Chicagog.

sensory pathways in the cervical sympathetic trunk by trial of electrical stimulation. With this point still not clear, we are left with the fact that the main recognized pathways of referred pain in angina were not touched, and yet the attacks are stopped by an operation which cuts two nerves, the superior cardiac and the main trunk between the superior and middle ganglions.

Bacon [16] reported a particularly severe case.

Case 16.—G. J., a man, aged 78, was a moderate drinker and a very hard worker. Three years ago, while climbing a hill, he was suddenly seized with a heavy feeling in the chest, over the precordium, which quickly disappeared on resting. Since then, similar attacks have followed exertion, with increasing frequency, and in March, 1923, they began to awaken him from sleep and became more frequent and severe at night. By April, severe attacks followed any excitement or exertion, such as coughing, eating or defecation; besides these, apparently spontaneous ones became very frequent. Rest and medical treatment gave only brief relief. His appetite disappeared, and vomiting frequently followed an attack. With increasing frequency and severity of attacks, 1/100 grain tablets of nitroglycerin were given under the tongue every half hour as a preventive, but without success. Slight temporary improvement followed the use of spirit of glyceryl trinitrate. June 13, after weeks in bed, the patient was receiving the equivalent of 1/100 grain of nitroglycerin under his tongue, by a trained nurse, every fifteen minutes night and day, whether sleeping or waking, from fifteen to twenty-five pearls of amyl nitrite during the twenty-four hours; 2 grains of sodium nitrite and 1/4 grain of morphin every three hours. In spite of this heroic dosage, he suffered eight severe attacks in six hours, during which the family thought the patient was dying and had the last sacraments of the church administered.

June 14, after the patient had passed a night of terrific suffering, under 1 per cent. procain local anesthesia, the left superior cervical ganglion was removed in toto.

August 14, two months after the operation, the patient was very cheerful and contented. His appetite was good and he slept well, he could also walk a mile a day without discomfort. He was not so short of breath as formerly. His greatest difficulty came during and after eating, when he had a feeling of pressure around the heart.

November 12, five months after operation, the patient reported that he was in better health than he had been during the last two years. He had gained 26 pounds (12 kg.), and his waist measure had increased 4 inches (10 cm.) in circumference. He frequently slept six hours at a stretch, and ate everything. Overeating, however, was followed by a feeling of fulness and pressure on the level of the sixth rib at its cartilage articulation on the right side. He had not experienced any pain in the heart region, but had an aching pain in both arms and hands when they were cold or he was cold, and when he became excited.

SUMMARY

Cutting the left superior cardiac branch of the cervical sympathetic and the main trunk below the ganglion has relieved the main condition in anginal attacks, and apparently the one from which the patients die in attacks. In a few cases, referred pains are not entirely relieved.

Jonnesco's original operation of removing all of the left cervical sympathetic tract and its three ganglions, and the first dorsal ganglion, has accomplished no more than this, for in Kappis' [8] case the character-

16. Bacon, J. H.: Left Superior Cervical Sympathectomy Under Local Anesthesia in Angina Pectoris, J. A. M. A. **81**:211 (Dec. 22) 1923.

istic attacks of angina recurred after four months. Jonnesco called attention in his original article to the need of bilateral removal, and it is possible in our own cases with incomplete relief of all symptoms that a bilateral operation would have brought complete relief. Jonnesco's patient declined the right sided operation because he was relieved without it. With our all too slight knowledge of the pathways from brain and cord to heart and their complete function, it is not right to condemn the procedure because of its failure in a small number of cases, at least until the similar operation has been done on both sides.

It seems clear to us that our operation removes the main cause of angina. This is based on the supposition (1) that angina is due primarily to spasm of the aorta but it may be also of the coronaries, for they are supplied by the same nerve systems of the sympathetic. The spasm is most commonly in the aorta but it may occur alone in the coronaries; (2) that the superior cardiac nerve has a constrictor action, and cutting it prevents the spasm. Our right sided case shows that it is possible that the bilateral operation is necessary in some cases, for in that case an unusually severe attack produced pain also in the left side of the neck and left arm, and frequently in cases with mainly left sided radiations an unusually severe attack will cause right sided radiations; (3) Case 3, Series 1,[2] reported again as Case 9 in this series, after removal of the left superior ganglion one and one-half years after severing of the superior cardiac nerve and main trunk of the sympathetic below the ganglion, shows that heart pain distinguished from substernal pain which we are attributing to aorta spasm, is in some way influenced by this upper ganglion. From twelve attacks during the three weeks in bed before the second operation he has had none in the four weeks since although up and about. It may be through vagus connections, and herein lies what possibilities there are in the Wenckebach-Eppinger-Hofer theory, that there is a separate depressor nerve, which when cut relieves angina. The superior laryngeal and the vagus which are said to give rise to this depressor nerve in man as they do in some animals, both have connecting branches to the superior ganglion.

It would seem that our operation removes the cause of the anginal attack, and that the more elaborate operation of Jonnesco removes the cause and also removes other motor and sensory pathways.

The operation may have to be bilateral to relieve completely all pain.

Only in event of further investigation can the limits be defined. In the meantime sixteen more cases of relief by removal of the superior ganglion are herewith reported, including our own, with two deaths, and fourteen with relief of the main symptoms.

THE REACTION OF THE LIVER TO PHENOLTETRACHLORPHTHALEIN IN EARLY OBSTRUCTIVE JAUNDICE *

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Attempts to measure the functional powers of the liver by various tests have occupied a rather prominent position in recent medical literature. The phenoltetrachlorphthalein test during the last few years has given promise of being the most valuable of the various methods proposed. Abel and Rowntree,[1] in 1909, discovered that, except for traces which appeared in the urine, this dye was apparently eliminated only by the liver after subcutaneous or intravenous injection of the disodium salt. They also found that the substance was nontoxic to dogs, even when injected daily for several months in very large doses. They noticed, moreover, that subcutaneous injections of the dye had a prolonged laxative action. Several years later Rowntree, Hurwitz and Bloomfield,[2] Whipple, Mason and Peightal,[3] and Whipple, Peightal and Clark [4] undertook clinical and experimental investigations on the elimination of this dye by the liver and its applicability as a clinical test for liver function. Their method consisted in the intravenous injection of the disodium salt, and subsequent collection of the feces for the next forty-eight hours which were examined for their dye content. They found that approximately from 50 to 60 per cent. of the unchanged dye was excreted by the liver; and in agreement with the work of Abel and Rowntree that the first portion of the dye to be excreted was in a conjugated form. They also observed that a trace of the dye, especially when large doses were given, appeared in the urine in a conjugated and also in a free form. They felt, however, that the test was not easily applicable because of uncertainties in the collection of the feces, and also because in cases in which there was obstruction

* From the Snydacker Fund and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research.

1. Abel, J. J., and Rowntree, L. G.: J. Pharmacol. & Exper. Therap. **1**:231, 1909.

2. Rowntree, L. G.; Hurwitz, H., and Bloomfield, A. L.: Bull. Johns Hopkins Hosp. **24**:327, 1913.

3. Whipple, G. H.; Mason, V. R., and Peightal, T. C.: Bull. Johns Hopkins Hosp. **24**:207, 1913.

4. Whipple, G. H.; Peightal, T. C., and Clark, A. H.: Bull. Johns Hopkins Hosp. **24**:343, 1913.

to the outflow of bile the dye could not be recovered from the feces. McNeil [5] modified the test by recovering the duodenal contents by means of the Einhorn tube after injection of the dye. He concluded from his studies that the amount of dye eliminated was of little value, but that its appearance time was delayed in certain pathologic changes in the liver. Kahn [6] utilized the Lyon-Meltzer method of securing a flow of bile after intravenous injection of the dye. In 1922, Rosenthal [7] still further modified the test. His procedure was to inject the dye intravenously in a dosage of 5 mg. of the disodium salt per kilogram of body weight, and then to examine specimens of blood serum taken at various intervals after the injection for their dye content. He observed that in normal animals the dye almost entirely or entirely disappeared from the blood stream within one hour. On removal of parts of the liver or on injuring that organ by poisons, such as chloroform, he found that various amounts of the dye were retained in the blood stream for more than one hour after injection. He extended his observations to human beings, and reported that in many diseased conditions in the liver there was a retention of the dye in the blood stream. Moreover, he found no retention of the dye in the blood stream of dogs when injected shortly after ligation of the common duct. Ottenberg and Rosen [8] found complete retention of the dye in three of four patients who had complete biliary obstruction of long duration.

The experiments reported in this paper were undertaken with the view to determine the fate of phenoltetrachlorphthalein after intravenous injection, immediately after ligation of the common and cystic ducts and to correlate these findings with the results of the Van den Bergh [9] test. The cystic duct was to be tied to prevent excretion of the dye into the gallbladder by the liver.

We found it necessary to modify slightly Rosenthal's [10] technic. He injected 5 mg. of the disodium salt per kilogram of body weight and then took samples of the blood serum fifteen and sixty minutes after injection of the dye, and on the addition of 3 drops of 5 per cent. sodium hydroxid to 1 c.c. of the dye containing serum he was able to

5. McNeil, H. L.: J. Lab. & Clin. Med. **1**:822, 1915.

6. Kahn, Max: Phenoltetrachlorphthalein Estimation in the Duodenal Contents, J. A. M. A. **77**:41 (July 22) 1921.

7. Rosenthal, S. M.: J. Pharmacol. & Exper. Therap. **19**:385 (June) 1922; A New Method of Testing Liver Function with Phenoltetrachlorphthalein, J. A. M. A. **79**:2151 (Dec. 23) 1922; Bull. Johns Hopkins Hosp. **33**:432 (Dec.) 1922.

8. Ottenberg, Reuben; and Rosen, Samuel: Possible Application of Phenoltetrachlorphthalein Test to Obstructing Jaundice, J. A. M. A. **80**:1519 (May 26) 1923.

9. Van den Bergh, A. A. H.: Presse méd. **29**:441 (June 4) 1921.

10. Rosenthal, S. M.: Proc. Soc. Exper. Biol. & Med. **21**:73, 1923.

read his test specimen against standard solutions of the dye of various strengths. We found this method very difficult, and capable of giving quite variable readings with various portions of the same specimen because of hemolysis, bilirubinemia or lipemia. We observed that the addition of one volume of acetone to one of the test serum precipitates the proteins sufficiently to leave on centrifugalization a water-clear supernatant fluid. The addition of 3 drops of 5 per cent. sodium hydroxid to this fluid shows the minutest traces of the dye, amounts of the dye which were usually not demonstrable by the Rosenthal method in our hands. The white precipitate does not change color on the addition of 5 per cent. sodium hydroxid. This method is, moreover, much more accurate with respect to the actual colorimetric readings in all concentrations of the dye because of the absence of opalescence in the test specimen. A permanent standard for reading the values of the dye in acetone treated serum was made up according to the Rosenthal [10] method, using distilled water alone as the diluent, except that, for each reading, the standard solution was made twice as strong and then diluted with an equal volume of acetone so as to give a color comparable to that of the test serum. After a month the acetone standard began to fade slightly.

It is to be noted that there is some confusion in the literature with respect to the use of the words plasma and serum. In some reports,[7] the term plasma is used when the protocols show clearly that serum and not plasma was actually meant.

In all of our experiments a preparation of the disodium salt of phenoltetrachlorphthalein was injected in an equal volume of physiologic sodium chlorid solution.

RESULTS

As a control experiment it was thought advisable to remove the liver entirely from the circulation in dogs, to inject phenoltetrachlorphthalein intravenously and to observe the blood stream for its content of the dye at various periods. The results of these experiments are tabulated in Table 1. A typical protocol (Dog 4, Table 1) is given of these experiments. It was thought advisable, moreover, to remove the kidneys, as it has been shown that when an excessive amount of the dye was injected the kidneys excreted a portion of the excess.

PROTOCOLS OF EXPERIMENTS

EXPER. 1.—Dog 4 (Table 1), female, mongrel, weight, 17 kg., under anesthesia of 1 grain of morphin, the right carotid artery was cannulated and a specimen of blood removed. The abdomen was incised in the midline. Ether anesthesia was then begun. The portal vein and hepatic artery were tied off; the liver was dissected from the inferior vena cava and the hepatic vein; these

vessels were ligated and the liver extirpated. Ether was discontinued. One hundred milligrams of the dye was injected intravenously. Specimens of blood taken from the carotid artery at the end of fifteen minutes, and from the left side of the heart at the end of one hour contained more dye than could be read on the colorimeter.

From Table 1 it may be seen that after intravenous injection of phenoltetrachlorphthalein after previous extirpation of the liver and kidneys the dye was apparently completely retained in the blood stream.

A series of experiments on five dogs was then performed in which

TABLE 1.—*Injection of Phenoltetrachlorphthalein After Extirpation of the Liver and the Kidneys*

Dog	Weight in Kg.	Mg. of Dye Injected	Time in Minutes Dog Lived after Injection of Dye with Liver and Kidneys Out	Amount of Dye in Blood of Left Heart at End of Experiment
1	18	250	32	40%*
2	10	100	22	40%
3	22	250	38	40%
4	12	100	67	40%
5	17	200	16	40%

* More than 40 per cent. of dye cannot be demonstrated in the blood serum by any technic now in use, irrespective of the amount of dye injected.

TABLE 2.—*Injection of Phenoltetrachlorphthalein After Removal of Kidneys and Ligation of the Common and Cystic Ducts*

Dog	Weight in Kg.	Mg. of Dye Injected	Amount of Dye in Blood after 1 Hour	Van den Bergh of Blood at End of 1 Hour	Dye in Hepatic Duct at End of Experiment	Dye in Gallbladder at End of Experiment	Remarks
1	17	150	0	.....	++++	++++	Cystic duct not ligated
2	16	250	0	Indirect in 15 minutes, direct in 60 minutes	0	0	
3	15	150	0	Faint indirect?	0	0	
4	17	250	7%	Very strong indirect	2%	0	Four times normal dose
5	8	120	9%	Direct ++++	Trace	0	Ten days after ligating the common duct

the kidneys were removed and the common and cystic ducts ligated. Into these animals the dye was injected intravenously, in dosages varying from 5 to 10 mg. per kilogram of body weight. In these experiments the blood was examined by the Van den Bergh test, and for its dye content. At the end of the experiment the bile of the hepatic duct and the various organs and tissues of the body were tested for the presence of the dye (Table 2). A typical protocol of the five experiments is appended (Dog 2, Table 2).

EXPER. 2.—Dog 2 (Table 2), yellow, male mastiff, weight 16 kg., was anesthetized with ether and 1 grain of morphin. The kidneys were removed and the common and cystic ducts ligated. Two hundred and fifty milligrams of

the disodium salt of phenoltetrachlorphthalein was injected into the right jugular vein. The blood at the beginning of the experiment gave a negative Van den Bergh reaction. Fifteen minutes after injection there was a faint indirect Van den Bergh reaction in the blood, and at the end of one hour there was a positive direct Van den Bergh reaction. At the end of fifteen minutes there was practically none of the dye in the circulation. At the end of one-half hour no dye whatever was demonstrable. The animal was allowed to live two hours after injection of the dye. It was found at necropsy that the lymphatics at the hilum of the liver were of a distinct yellow color. The thoracic duct was enlarged and quite golden yellow. The bile from the proximal portion of the hepatic duct contained none of the dye. The cut section of the liver did not change color on application of 5 per cent. sodium hydroxid. Portions of the liver, heart and lungs were boiled with 5 per cent. sodium hydroxid and 5 per cent. hydrochloric acid and none of the dye was demonstrated.

To test the possibility of the dye having broken down in the liver, with a resultant liberation of chlorin, a portion of the liver was examined quantitatively for its free chlorids by the urine chlorid method of Vollhard and Harvey,[11] after incubation at 37.5 C. (for twenty-four hours) in distilled water in the presence of thymol dissolved in ethyl alcohol. This was not found to be higher than that of an equal portion of normal dog liver. Because of the small amount of chlorids present and the resulting high percentage of error, it is felt that no conclusions are justified from these chlorid determinations.

The results of these experiments were considered exceedingly interesting, since one of us [12] had shown that in similar experiments, without the injection of the dye, an indirect reaction by the Van den Bergh method was seldom obtained before two hours after tying the biliary ducts. He also found that the indirect Van den Bergh reaction alone was obtained during the first day after ligation of the common and cystic ducts and after this the direct reaction appeared.

After this, similar experiments were then performed on five dogs in which the thoracic duct was also cannulated and the lymph tested by the Van den Bergh method and for its dye content. These experiments, which are presented briefly in Table 3, indicate that with the exception of the experiment on Dog 5, practically none of the dye appeared in the lymph stream. In this animal, twenty-two days elapsed between the ligation and section of the common duct and the injection of the dye. The animal was severely jaundiced, and after the introduction of the dye it was found that the lymph very soon showed the presence of the dye in a concentration almost equal to that in the blood stream. Because of this experiment, it was felt that in chronic obstructive jaundice, where there is microscopic evidence of severe damage to the liver cells, the liver, being unable to remove all of the dye brought to it by the blood stream, turns over part of it into the lymph channels. We believe, however, that there is no parallelism between

11. Harvey, S. C.: The Quantitative Determination of the Chlorids in the Urine, Arch. Int. Med. **6**:12 (July) 1910.

12. Bloom, W.: Bull. Johns Hopkins Hosp. **34**:316 (Sept.) 1923.

the reaction of the liver in early obstructive jaundice to bilirubin and to phenoltetrachlorphthalein. For, in acute experiments, the former is present in increasingly greater amounts in the thoracic duct, while the latter hardly appears at all, even when very large doses are injected intravenously.

The liver tissue of five animals in the preceding experiments, in which the injected dye disappeared from the blood stream, was subjected to the action of boiling 5 per cent. sodium hydroxid and 5 per cent. hydrochloric acid, and of reducing and oxidizing agents, such as dilute iodin, potassium permanganate, hydrogen peroxid and nascent hydrogen. It was impossible to recover dye after any of these procedures. Nor was it possible on repeated trials to demonstrate a higher chlorid content in these than in normal livers.

In another series of experiments, 5 gm. each of the following organs from four normal dogs were minced and suspended in 4 c.c. of physio-

TABLE 3.—*Injection of Phenoltetrachlorphthalein After Ligation of Common and Cystic Ducts and Cannulation of Thoracic Duct and Removal of Kidneys*

Dog	Weight in Kg.	Mg. of Dye Injected	Time Lapse Between Ligating Common Duct and Injection of Dye	Phenoltetrachlorphthalein in Blood		Phenol-tetrachlor-phthalein in Lymph
				End of 30 Minutes	End of 60 Minutes	
1	10	70	0	0	0	0
2	5	40	2 hours	+	0	Trace
3	40	250	2 hours	3%	0	0
4	5	50	5 hours	+	0	0
5	10	100	22 days	5%	4	3%

logic saline solution. To each specimen one drop of 0.5 per cent. of disodium phenoltetrachlorphthalein and two drops of thymol solution in ethyl alcohol were added and incubated at 37.5 C. (99.1 F.). The specimens were examined for the presence of the dye after one, two, six and eighteen hours. The organs tested were the striped muscle, heart, lung, kidney, liver, spleen, and large bowel. It was found that at the end of twenty-four hours the heart had so changed the dye that practically none of it could be demonstrated after hydrolysis with 5 per cent. sodium hydroxid and 5 per cent. hydrochloric acid. The lung and spleen also decolorized the dye more than the liver. The striped muscle seemed to have no effect on the dye.

SUMMARY OF EXPERIMENTAL RESULTS

The experiments reported in the foregoing showed that in the absence of the liver and kidneys the dye remained in the circulation as long as the animals lived. After tying the common and cystic ducts, the liver was able to remove large quantities of phenoltetrachlorphthalein

from the circulation. The dye in these experiments was not excreted in significant quantities by the liver into the bile passages, and it did not appear in the lymph of the thoracic duct. We were unable to recover the dye from these livers by treating them with hydrochloric acid, sodium hydroxid, oxidizing or reducing substances, so that we believe that the liver cells either combined very intimately with the dye or else changed it into a more or less stable, colorless compound or compounds. It was thought that if the phenoltetrachlorphthalein was broken down in the liver, some of its chlorin might be liberated. We have no evidence to support this theoretical liberation of chlorin. We

TABLE 4.—*Series of Patients Suffering from Various Diseases*

Cases	Number of Cases	Number Showing Increase in Indirect Van den Bergh or in Direct when Positive	Number Showing Abnormal Dye Retention at End of 1 Hour	Average Amount of Dye in Urine	Untoward Reactions: Number Showing Thrombosis	Untoward Reactions: Chills	Untoward Reactions: Sterile Abscesses or Local Reaction	Total Number of Cases Showing Untoward Reaction
Group 1. Miscellaneous without liver diseases	8	8	0	Less than 0.5 mg.	2	1	—	2
Group 2. Miscellaneous with liver disease, carcinoma, amyloid, syphilis, tuberculosis	5	5	3 (3-7%)*	Less than 0.5 mg.	1	—	1	2
Group 3. Cardiac decompensation (chronic passive congestion of liver)	4	4	4 (4-9%)	Less than 0.5 mg.	1	—	1	2
Group 4. Acute and chronic cholecystitis with or without cholelithiasis	4	4	0	0.5 mg.	—	1	2	2
Group 5. Laënnec's atrophic cirrhosis of liver	3	3	3 (7-8%)	Less than 1 mg.	—	—	1	1
Group 6. Obstructive jaundice								
Due to stone	2	1	1 (2%)	Less than 1 mg.	—	—	1	1
Due to cholangitis	1	1	1 (4%)					
Total	27	26	12		4 (14.8%)	2 (7.4%)	6 (22.2%)	10 (37.0%)

Amount of dye injected routinely: 5 mg. per kilogram of body weight.
* The percentage figures in this column indicate the amount of dye retention.

have been unable to determine the fate of the dye in the liver of the dog in early obstructive jaundice. The finding that the intravenous injection of phenoltetrachlorphthalein causes a very rapid appearance of the indirect and direct Van den Bergh reactions is considered more fully when the results of our clinical studies are discussed.

CLINICAL RESULTS

A series of patients suffering from various diseases, and a number of apparently normal persons were injected with 5 mg. of the dye per kilogram of body weight and at the end of one hour the blood was

examined by the Van den Bergh test and for its dye content. We noticed that the fifteen minute specimen added nothing to the information obtained from the hour specimen of serum.

Here again, the findings first noticed in the experiments on the dogs in Table 2 were confirmed and extended. It was seen that even in normal persons there was a transient hyperbilirubinemia, as evidenced by a quantitative increase of the indirect Van den Bergh reaction. This hyperbilirubinemia, which usually disappeared within twenty-four hours, seemed to bear no relation to the severity of impairment of liver function as measured by the dye retention. We believe that the transient bilirubinemia subsequent to the injection of phenoltetrachlorphthalein was due to the fact that the liver, having an apparently specific, excretory action toward the dye after it had been injected intravenously, cannot excrete bilirubin or its precursors which are brought to it until all of the dye has been eliminated. We have no evidence that this transient bilirubinemia is indicative of anything more than a temporary functional blockade of the bilirubin excreting power of the liver. As a control experiment, phenoltetrachlorphthalein was added to blood serum in vitro; it had no apparent effect on the Van den Bergh test. The dye alone, moreover, did not give a color reaction with Ehrlich's diazoreagent.

As may be seen from Table 4, 14.8 per cent. of the patients injected with the dye developed thromboses at the site of injection, 22.2 per cent. local reactions, 7.4 per cent. chills; a total of 37 per cent. untoward reactions were met with in this series. Small traces of the dye in a free or a conjugated form were found in the urine of normal persons as well as patients suffering from various types of liver disease. The total amount of the dye in the urine was never more than 3 mg., that is, less than 1 per cent. of the total dye injected. In none of our cases with complete biliary obstruction have we ever found a dye retention greater than 4 per cent. This is in agreement with our findings in experimental animals and at variance with the results of Ottenberg and Rosen.[8]

CONCLUSIONS

1. In the absence of the liver and kidneys, phenoltetrachlorphthalein continued to circulate in the blood stream.

2. The intravenous injection of phenoltetrachlorphthalein in the early stages of obstructive jaundice in dogs caused an earlier appearance of both the indirect and the direct Van den Bergh reactions that was noticed in control animals.

3. All attempts to recover the dye from the liver in obstructive jaundice were unsuccessful.

4. Dog liver decolorized phenoltetrachlorphthalein less than the heart, lung, and spleen in vitro.

5. In early obstructive jaundice, phenoltetrachlorphthalein appeared in minute amounts or not at all in the lymph of the thoracic duct. In chronic obstructive jaundice, however, the dye was present in large amounts in the lymph of the thoracic duct.

6. The intravenous injection of phenoltetrachlorphthalein produced in the human being a transient hyperbilirubinemia, as evidenced by the Van den Bergh test.

7. The high incidence of thromboses and other untoward reactions after the injection of phenoltetrachlorphthalein, is a serious objection to the execution of this test as a clinical method.

8. In no case of long standing complete biliary obstruction have we noticed a greater dye retention than 4 per cent. in the blood serum.

9. A new method is given for the accurate determination of small amounts of phenoltetrachlorphthalein in blood serum.

MITRAL STENOSIS WITHOUT RHEUMATIC FEVER IN NORTH CHINA *

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Ever since the etiologic relationship between rheumatic fever and stenosis of the mitral valve was established half a century ago by Hayden,[1] Fagge,[2] and Duckworth,[3] these two conditions have been closely associated in the minds of European and American students of

TABLE 1.—*Relative Incidence of Mitral Stenosis in Peking and Elsewhere*

Hospital	Years	Medical Patients	Cases of Mitral Stenosis	Per Cent. Mitral Stenosis
St. Bartholomew's, London	1913	1,996	50	2.5
Johns Hopkins, Baltimore	1914	1,422*	17	1.2
P. U. M. C., Peking	July 1, 1921 to Jan. 31, 1924	2,694	39	1.45

* White patients only are included. Among 311 negro patients there were no cases of mitral stenosis.

TABLE 2.—*Relative Incidence of Rheumatic Fever and Infection Arthritis in Peking and Elsewhere*

Hospital	Years	Medical Patients	Fever		Arthritis		Totals	
			No.	%	No.	%	No.	%
			Rheumatic		Infectious			
St. Bartholomew's, London	1913	1,996	105	5.26	1	0.05	106	5.3
Johns Hopkins, Baltimore	1914	1,422*	...	....	44†	3.1	44	3.1
P. U. M. C., Peking	July 1, 1921 to Jan. 31, 1924	2,694	6‡	0.2	6	0.2	12	0.4

* White patients only are included.
† These include cases of rheumatic fever.
‡ None of these was as severe as the typical cases of rheumatic fever seen in America.

heart disease. It is interesting to discover, therefore, that in North China, where typical rheumatic fever is almost unknown, mitral stenosis is the most common heart lesion, and its incidence is comparable to that in western countries. Table 1 shows the prevalence of mitral stenosis in our records as compared with those of St. Bartholomew's Hospital, London, for 1913, and of the Johns Hopkins Hospital, Bal-

* From the Department of Medicine, Peking Union Medical College.

1. Hayden: Diseases of the Heart and the Aorta, Dublin, 1875, p. 964 (quoted by Duckworth).

2. Fagge, C. H.: On the Murmurs Attendant upon Mitral Contraction, Guy's Hosp. Rep., 3rd Series, **16**:247, 1871.

3. Duckworth, D.: On the Aetiology of Mitral Stenosis, St. Barth's. Hosp. Rep. **13**:263, 1877.

timore, for 1914. In contrast to this, Table 2 shows the relative incidence of rheumatic fever and infectious arthritis in the same hospitals.

This paper deals with thirty-nine cases of mitral stenosis occurring in Chinese patients. These include all of the definitely diagnosed cases of this condition which have been admitted to our wards since the new hospital was opened. We have included only patients which presented the typical physical signs of the disease, namely, a presystolic thrill and a presystolic rumbling crescendo murmur (or its equivalent in the presence of auricular fibrillation) localized to the apical region of the heart. No cases of marked aortic insufficiency are included, in which the apical murmur could be mistaken for a so-called Austin Flint murmur.

RHEUMATIC ANTECEDENTS

We have reviewed these cases particularly from the standpoint of their etiologic relationship to rheumatic fever, acute arthritis and chorea, the three conditions which were termed by Duckworth "rheumatic antecedents." Of the thirty-five cases in which our records make a definite statement, only one gave a history of rheumatic fever, and one a history of chorea; while fourteen others gave a history of joint

TABLE 3.—*Relative Incidence of Rheumatic Antecedents in Cases of Mitral Stenosis*

Cases Reported by	No. of Cases	Rheumatic Antecedents				
		Rheumatic Fever	Chorea	Joint Pains	Total	Per Cent.
Duckworth	80	47	4	5	56	70.0
Meleney and Kellers	35*	1	1	14	16	45.7

* In four cases there was no record whether or not there had been joint pains.

pains of varying intensity. The patient with a history of rheumatic fever stated that fifteen years ago, at the age of 12, she had had fever with painful, red and swollen joints. This had recurred five or six times since then. Of the fourteen patients listed as having had joint pains, five had had fever (two of them slight) and in all these multiple joints (four or more) had been involved. Ten patients in all had multiple joint pains; one had three joints involved, three had only two. Swelling of joints occurred in only one patient. Redness of joint regions never occurred. The patient with a history of chorea stated that he had had it at 12 years of age, five years before admission, and that it lasted for one year.

These cases present a strong contrast to those of Duckworth.[3] Of his eighty cases of mitral stenosis fifty-six patients had had rheumatic

antecedents. Of these, forty-six had had one or more typical attacks of rheumatic fever. Four had had chorea alone and one both chorea and rheumatic fever. Three had had "rheumatic pains," one "rheumatic gout" and one osteoid arthritis. Table 3 shows the contrast between these patients and ours.

In the absence of more definite rheumatic antecedents in these cases we have searched for other etiologic factors, but no greater tendency to other known infections has appeared than is met with in the general taking of histories. For instance, only ten of our patients gave a history of sore throats, and of these, only three had had joint pains as well. Five patients had had toothache, and three of these had had joint pains also. Four had had otitis media, and one of these joint pains also. Thirteen had a history of smallpox, twelve of measles, five of typhoid or some similar type of fever, and four of gonorrhea, with joint pains in one of these.

PHYSICAL EXAMINATION

The general physical examination revealed no more definite evidence of sources of infection than did the history. In twelve patients the tonsils were enlarged; in eight of these they were cryptic, and in two inflamed as well. In fifteen the teeth showed pyorrhea; in only five was there caries, and in three others, root abscesses. Three patients were slightly deaf in one ear; one of these had a thickened and retracted ear drum. One patient had tuberculosis of the lungs and of the humerus. In one the history and physical signs pointed to a gonococcal salpingitis.

In other features our series of cases corresponds closely to those reported elsewhere. Although there are only fourteen female patients in our series as against twenty-five males, this inversion of the usual proportion in mitral stenosis is practically abolished when it is considered that our total number of male medical patients has outnumbered the females by 3.2 to 1. This correction brings the theoretical percentage to: females 62.7 per cent., males 37.3 per cent. This corresponds closely to the ratio in the cases collected by Duckworth: females 67.3 per cent., males 32.6 per cent.

AGE GROUPS

The age groups also correspond fairly closely to those reported by Duckworth, as shown in Table 4. The highest incidence in our series, however, is reached in the second decade, whereas in Duckworth's series, the third and fourth decades had the highest incidence and were nearly equal. Table 4 also shows the age of onset of symptoms in our series. It will be seen that the second decade has by far the largest incidence of onset, increasing over the simple age incidence at the

TABLE 4.—*Age Distribution of Cases of Mitral Stenosis*

Age, Decade	Duckworth's Cases	P. U. M. C. Cases	P. U. M. C. Onset of Symptoms
1	0	1	1
2	14	12	18
3	24	9	8
4	23	8	4
5	13	6	5
6	5	3	3
7	1	0	0
Total cases	80	39	39

expense primarily of the fourth decade. The close correspondence of the "incidence" and "onset" columns in the fifth and sixth decades may be due either to a late onset of the pathologic process, or to a very mild lesion of long standing which first gave symptoms only when the myocardium began to grow old. This latter possibility is supported by the necropsy findings in Case 35, which are mentioned later.

ELECTROCARDIOGRAPH FINDINGS

In connection with the diagnosis of our cases of mital stenosis it is interesting to note the abnormalities shown by the electrocardiograph. These were as follows:

Of thirty-five cases on whom records were taken, there was right ventricular preponderance in eighteen cases; auricular fibrillation in thirteen cases; auricular fibrillation constantly in six cases.

Of the twenty-nine cases in which the P wave was present, there was notching of the P wave in seventeen cases; flat top P wave in four cases; inverted P wave in Lead 3 in eight cases; P-R interval above 0.20 seconds in seven cases; deflection of P wave above 0.2 millivolts in fourteen cases.

It is noteworthy that, in two instances in our series, two cases were present in the same family. Cases 25 and 28 are brother and sister. Both have a history of vague joint pains. Again, Case 34 is the mother of Case 1. In neither case were any "rheumatic antecedents" known.

Out of thirty-seven patients in which a blood Wassermann test was performed, four were positive (4 plus). Three of these cases had no "rheumatic antecedents." The other patient had joint pains lasting for three months at the time of onset of heart symptoms four years ago. No opportunity has been offered to check up the diagnosis on these cases at necropsy.

One of our patients had paralysis of the left recurrent laryngeal nerve, a complication of mitral stenosis which has often been reported [4].

4. Garland, J., and White, P. D.: Paralysis of the Left Recurrent Laryngeal Nerve Associated with Mitral Stenosis. Arch. Int. Med. **26**:343 (Sept.) 1920.

TABLE 5.—*Analysis of Thirty-Nine Cases of Mitral Stenosis in Peking*

Case	Sex	Age	Age at Onset	Rheumatic Fever	Chorea	Joint Pains	Other Infections	Wassermann	Electrocardiogram: Right Ventricle Preponderance	Auricular Fibrillation	Notched P	Flat Top P	Inverted P Lead 3	P-R Intervals in Seconds	Deflection of P in Millivolts	Remarks
1	M	10	10	0*	0	0	†4, 8	0	+	0	+	0	+	0.14	0.25	
2	M	14	12	0	0	0	4, 8	0	+	0	+	0	+	0.18	0.3	
3	F	15	13	0	0	+	1, 5, 7	0	0	+	0	+	0	0.2	0.15	Died; no necropsy
4	M	15	14	0	0	+	0	0	0	0	+	0	0	0.24	0.25	Died; necropsy
5	M	16	11	0	0	+	4?, 6?	0	—	—	—	—	—	—	—	
6	M	17	14	0	0	+	0	0	+	0	+	0	0	0.18	0.25	Died at home
7	M	17	17	0	0	0	4?	0	+	0	0	0	0	0.16	0.5	
8	M	17	11	0	0	0	1, 5, 9	0	—	—	—	—	—	—	—	
9	M	17	12	0	+	0	3, 9	0	+	0	+	0	0	0.16	0.1	
10	M	18	17	0	0	+	2	0	0	+	+	0	0	0.24	0.15	
11	M	18	17	0	0	—	7, 8	0	+	0	0	+	+	0.16	0.3	Paralysis left recurrent laryngeal nerve
12	F	20	20	0	0	0	2	0	0	0	+	0	+	0.16	0.3	
13	M	20	18	0	0	0	3, 7	0	+	+	0	+	0	0.24	0.1	
14	M	22	17	0	0	+	0	0	0	0	+	0	0	0.2	0.2	
15	M	24	24	0	0	—	3	0	0	0	0	+	0	0.12	0.25	
16	F	24	20	0	0	+	3, 4	++++	—	—	—	—	—	—	—	
17	M	27	27	0	0	0	3	0	0	0	+	0	0	0.24	0.5	
18	M	27	12	+	0	0	7, 8	0	+	+	0	0	0	0.24	0.3	
19	F	28	25	0	0	+	2, 4, 7	0	+	+	—	—	—	—	—	
20	F	28	23	0	0	+	2, 4	0	+	0	+	0	0	0.2	0.15	
21	F	28	28	0	0	+	0	0	0	+	+	0	+	0.18	0.1	
22	F	29	17	0	0	0	1, 2, 4	0	0	0	+	0	+	0.22	0.2	
23	M	32	24	0	0	+	0	0	+	+	—	—	—	—	—	Died; necropsy
24	F	33	23	0	0	0	4, 7	0	0	0	+	0	0	0.16	0.25	
25	M	35	22	0	0	+	3, 7, 8	0	+	+	+	0	0	0.2	0.3	
26	M	35	34	0	0	0	0	0	‡	0	+	0	0	0.18	0.25	
27	F	36	16	0	0	0	0	++++	+	0	0	0	0	0.16	0.3	
28	F	38	35	0	0	+	0	0	0	0	0	0	0	0.2	0.2	
29	M	40	36	0	0	—	7, 9	—	0	+	—	—	—	—	—	
30	F	41	37	0	0	0	1, 3, 4, 7, 8, 11	0	+	+	+	0	0	0.14	0.1	
31	M	41	41	0	0	0	3, 9, 10	++++	+	0	0	0	+	0.16	0.15	Died; no necropsy
32	M	42	41	0	0	—	3, 8, 9, 10	0	0	0	0	0	0	0.12	0.5	
33	M	43	43	0	0	0	4, 8	++++	0	Flutter	—	—	—	—	—	
34	F	44	24	0	0	0	0	0	+	0	0	0	0	0.16	0.2	
35	F	47	47	0	0	0	3, 7	0	0	+	—	—	—	—	—	Died; necropsy
36	F	49	43	0	0	0	3, 4, 7, 8	0	+	0	0	0	0	0.24	0.15	
37	M	53	53	0	0	0	3	0	+	0	+	0	+	0.18	0.2	
38	M	55	55	0	0	0	3, 4, 9, 10	0	0	+	—	—	—	—	—	
39	M	59	56	0	0	+	3, 8, 10	0	0	+	0	0	0	....	0.15	
Totals	M. 25 F. 14	Av. 30	Av. 26	1	1	14		4	18	13	17	4	8			

* +, positive; 0, negative; —, not known.

† In "Other Infections" column: 1, otitis media; 2, toothache; 3, smallpox; 4, measles; 5, chickenpox; 6, mumps; 7, malaria; 8, dysentery; 9, typhoid-like fever; 10, gonorrhea; 11, tuberculosis.

‡ Lead 1 of electrocardiogram unsatisfactory.

NECROPSY FINDINGS

Of the six known deaths in our series three cases came to necropsy. In all of these the diagnosis of mitral stenosis was confirmed. The character of the stenosis in all three cases was that of a scarring as the apparent result of a chronic inflammatory process, with thickening of the valve flaps, thickening and shortening of the chordae tendineae and fibrosis of the papillary muscles and endocardium adjacent to the valve. The left auricle was hypertrophied and dilated in all three cases. In one patient (Case 35) a broken thrombus was found in the left auricle which may have been the cause of death. The aortic valve also was definitely distorted and stenotic in two of the three cases (Cases 23 and 35).

Table 5 gives in detail the joints dealt with in this paper.

COMMENT

It has always been recognized that a certain number of cases of mitral stenosis have no clinical relationship to rheumatic fever or to any other definite infectious process. Such cases, however, have uniformly shown the same pathology at necropsy as cases in which there was definite association with rheumatic antecedents. The same is true of our cases, both clinically and pathologically. We cannot say, therefore, that these cases are not fundamentally the same as those occurring in association with rheumatic fever. They are apparently the result of some mild inflammatory process of a progressive nature which acts primarily on the most sensitive portion of the endocardium. It has never been established, however, that the so-called rheumatic endocarditis is caused by a specific micro-organism, although a nonhemolytic form of the streptococcus has been under suspicion. Our cases emphasize the obscurity of entrance and action of the infectious agent, whatever it may be, and call attention to the possibility that a variety of organisms or toxins may produce the same pathologic picture.

The scarcity of rheumatic fever in Peking is as striking as is the presence of mitral stenosis. We can offer no satisfactory explanation for this. Nowhere in China is rheumatic fever as prevalent as in America or England. Possibly more cases are seen in the damp climate of the Yangtze Valley than in the dry regions of North China; but even in Shanghai and Changsha only a few cases are met with. Certainly the climate is not a great factor in China, although Poynton and Paine[5] consider it so in England. Perhaps more important is a racial tendency of the Chinese to be less susceptible than occidentals to the type of pathologic change to which arthritis belongs. Arthritis

5. Poynton, F. J., and Paine, A.: Researches on Rheumatism, New York, 1914, p. 392.

deformans, arteriosclerosis and gout are relatively rare among the Chinese. Whether this difference in susceptibility lies in the fact that the Chinese diet is primarily vegetarian, or whether some more intricate factors are responsible, we are not prepared to say.

SUMMARY

1. Mitral stenosis is the most common valvular disease of the heart in Peking, although rheumatic fever is very rarely seen.

2. Thirty-nine cases from the medical clinic of the Peking Union Medical College Hospital are reviewed. In the histories of these patients only one case of rheumatic fever, one case of chorea and fourteen cases of "joint pains" occurred.

3. No other infectious diseases appear to be especially involved in these cases of mitral stenosis.

4. In sex and age incidence, in electrocardiographic phenomena and in the pathology of three cases which came to necropsy, these cases are similar to the cases of mitral stenosis of rheumatic origin seen in Europe and America.

THE CONCENTRATING CAPACITY OF THE KIDNEY *

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A capacity for concentration must be conceded to the kidney, because most of the substances which it excretes are found to be in a state of higher concentration in the urine than in the plasma. The mechanism through which this concentration is accomplished cannot easily be conceived of as a process in which the kidney remains passive. It seems necessary to postulate that work is done by the renal tissue in converting a dilute plasma solution into a relatively concentrated urinary solution. On this account there has always been a special interest attached to observations dealing with the manner in which such an active function is affected by changes in the environment and structure of the kidney, though it must be admitted that there is as yet no unanimity of opinion regarding the factors which influence the renal concentrating capacity, nor any clear conception as to the method by which the concentration is produced.

Since 1909, when Ambard and Papin [1] published some data on this subject, the idea that there is a fixed upper limit to the concentrating capacity of the kidney for each urinary constituent has been widely accepted, and although it cannot be maintained that either their results, or the results of other authors which have since been published, constitute a proof that such maximum limits actually exist, it is at least the experience of all observers that certain high concentrations are rarely exceeded and then only under extreme conditions. This circumstance may be taken as an indication that the conditions under which these hypothetic limits are approached are those which are best adapted for an investigation of the factors which influence the concentrating capacity of the kidney. The general nature of these conditions is obvious from the fact that the concentration of any solid substance in the urine increases in direct proportion to every increase in the rate at which it is excreted, and decreases in direct proportion to every increase in the rate at which water is excreted. The highest concentrations are therefore to be expected when the rate of excretion of the substance in question is most increased and the volume of urine is at the same time reduced to a minimum.

Preliminary experiments were carried out in 1915, to obtain some general data with regard to the concentrating capacity of the kidney,

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1. Ambard and Papin: Arch. internat. de physiol. **8**:437, 1909.

and in particular to determine which of the various urinary constituents should be selected for special study. It was foreseen that at a later date we might wish to measure the effect which a change in the concentration of a substance in the blood might have on the concentration at which it was eliminated in the urine. This requirement excluded certain substances which might otherwise have been chosen, because the methods available for their determination in blood did not have the reliability necessary for our purpose. It was also necessary to choose those substances whose rate of excretion in the urine could be markedly increased. Urea, sodium chlorid, and sodium acid phosphate can all be taken in considerable quantity and in high concentration. They were given to normal persons whose urine volume had been decreased by abstention

TABLE 1.—*Decrease in the Rate of Water Excretion, Combined with Increase in the Rate of Excretion of the Substance Studied*

Name	After 30 Gm. of Urea in 15 per Cent. Solution: Urea, per Cent.	After 10 Gm. Sodium Chlorid in 5 per Cent. Solution: Sodium Chlorid, per Cent.	After 10 Gm. Sodium Acid Phosphate $4H_2O$ in 5 per Cent. Solution: Sodium Acid Phosphate, per Cent.
I.	4.38	1.78	1.77
W.	3.39	1.69	1.80
Mc.	3.41	1.50	1.82
S.	3.55	1.57	1.64
M.	4.06	2.25	1.29
B.	4.36	1.42	1.77
K.	4.85	1.74	2.07
Sh.	3.92	1.36	1.52
Average concentration	3.98	1.66	1.71
Average rate of water excretion	596 c.c.	364 c.c.	222 c.c.
Average rate of substance excretion	23.2 gm.	6.04 gm.	3.75 gm.

from water, and who were taking a diet which was low in sodium chlorid and in water. Other details of the experiments are given in a previous paper.[2] Thirty grams of urea were given in the first experiment, 10 gm. of sodium chlorid in the second, and 10 gm. of sodium acid phosphate in the third. In each case the solid was dissolved in 200 c.c. of water and ingested at 8 p. m. The concentrations found in the twelve hours' night urine (8 p. m. to 8 a. m.) are given in Table 1.

These concentrations are high, and in some cases approach the highest that have been observed. But it will be noted that with this method, in which the conditions are designed to produce a concomitant reduction of urine volume and increase of excretion of solids, we were not successful in maintaining the urine volume at a minimum, for there is a wide difference between the 596 c.c. excreted when urea was administered and the 222 c.c. eliminated after phosphate ingestion. It seemed

2. Addis T., and Foster, M. G.: Specific Gravity of Urine, Arch. Int. Med. **30**:555 (Nov.) 1922.

possible, therefore, that fluid restriction alone might be as effective as the combined procedure. The same group of persons was therefore placed under the same conditions except that no urea, sodium chlorid or sodium acid phosphate was given, and the 200 c.c. of water in which these substances had been dissolved was also withheld. In this experiment, the concentration of all three substances was determined in the twelve hours' night urine (Table 2).

These concentrations are all considerably lower than those observed when the rate of excretion of the substance studied was increased, even though the rate of water excretion was much less than after urea or sodium chlorid had been given. It was therefore concluded that both fluid restriction and the administration of the substance selected for investigation was advantageous in studying the concentrating capacity of the kidney.

TABLE 2.—*Decreases in the Rate of Water Excretion Only*

Name	Urea, per Cent.	Sodium Chlorid, per Cent.	Sodium Acid Phosphate, per Cent.
L.	3.51	0.468	0.604
W.	1.47	0.485	0.380
Mc.	2.59	1.160	0.605
S.	2.60	1.064	0.567
M.	1.90	1.190	0.595
B.	2.57	0.856	0.845
K.	3.56	0.863	0.750
Sh.	1.41	0.285	0.295
Average concentration	2.45	0.706	0.580
Average rate of water excretion	243 c.c.		
Average rate of substance excretion	5.40 gm.	1.78 gm.	1.28 gm.

It will be noted (Table 1) that phosphate administration was followed by a urine volume which was even less than when no additional substance was given. On this account we carried out another experiment in which 10 gm. of sodium acid phosphate (NaH_2PO_4-4 H_2O) was taken and in this case determined the urea and chlorid as well as the phosphate concentrations. We were only able to obtain the cooperation of three of the subjects of the former experiments, but in all other respects the results are comparable (Table 3).

In spite of the low urine volumes the urea and chlorid concentrations are not so high as in the experiments in which urea and chlorid were taken. At this point we were deflected from our original purpose by our interest in the cause of the decrease in the rate of chlorid excretion which followed the ingestion of phosphate. Under other conditions this phenomenon is more pronounced than in the experiments we have given here, but no satisfactory explanation was obtained up to the time when the work was interrupted by the war. However, one other experiment was carried through, with the original group of subjects, in order to find whether still higher concentrations might not be attained if we

reduced the urine volume by giving phosphate while at the same time the rates of excretion of urea, chlorid and phosphate were increased by giving a mixture of all three substances. We were afraid that vomiting might be produced if we gave full doses of sodium chlorid and phos-

TABLE 3.—*Decrease in the Rate of Water Excretion with Simultaneous Increase in the Rate of Excretion of Sodium Acid Phosphate by the Administration of 10 Gm. Sodium Acid Phosphate*

Name	Urea, per Cent.	Sodium Chlorid, per Cent.	Sodium Acid Phosphate, per Cent.
I.	2.49	0.880	1.77
K.	2.49	0.736	2.07
Sh.	1.85	0.657	1.52
B.	2.76	0.851	1.80
Ba.	2.94	0.465	2.09
L.	2.21	0.556	1.54
Bo.	1.65	0.592	1.89
D.	1.16	0.552	1.67
Average concentration	2.19	0.66	1.79
Average rate of water excretion	227 c.c.		
Average rate of substance excretion	4.89 gm.	1.51 gm.	4.00 gm.

phate, so 5 gm. of each was given with 30 gm. of urea, all dissolved in 200 c.c. of water. The concentrations we found are given in Table 4.

Under these conditions the average concentrations are lower than when each substance is given alone, and in the case of chlorid and phosphate are even lower than when no chlorid or phosphate was given, because the increase in the rate of water excretion which the

TABLE 4.—*Decrease in the Rate of Water Excretion with Simultaneous Increase in the Rate of Excretion of Three Substances by the Administration of a Mixture of 30 Gm. of Urea, 5 Gm. of Sodium Chlorid and 5 Gm. of Sodium Acid Phosphate*

Name	Urea, per Cent.	Sodium Chlorid, per Cent.	Sodium Acid Phosphate, per Cent.
I.	4.00	0.863	0.655
W.	3.70	1.165	0.450
Me.	3.15	0.450	0.468
S.	3.27	0.878	0.526
M.	3.82	1.080	0.591
B.	3.70	0.440	0.626
K.	3.35	0.884	0.580
Sh.	3.65	0.379	0.459
Average concentration	3.58	0.761	0.544
Average rate of water excretion	618 c.c.		
Average rate of substance excretion	22.02 gm.	4.64 gm.	3.34 gm.

mixture induced was greater than the increase in the rate of excretion of chlorid and phosphate. The conclusion that we then reached was that the optimum conditions for a study of the concentrating capacity of the kidney for any substance could be met by restriction of fluids, and administration of that substance alone in a solution as concentrated as possible.

These experiments have been given in detail, because the results have a bearing on certain theories as to the mechanism underlying the concentrating capacity of the kidney.

One hypothesis which might be advanced would explain the difficulty which is experienced in obtaining concentrations which surpass a certain rather ill defined limit, and would also enable us to bring under one category the observed maximal concentrations of all urinary constituents. This conception has been expressed with the greatest clarity by Ambard [3] in the statement that the maximal concentrations for urea, sodium chlorid and glucose are approximately isotonic with one another. If this were an established fact it would be a basis for a generalization as to the limitation of the concentrations of substances in the urine. It would be plausible to suppose that the limitation consisted in the inability of the kidney to remove from the blood any more of a given substance when the osmotic pressure of that substance had surpassed a certain level in the urine, a level which was the same for all urinary constituents. But because this is a simple and satisfactory theory, it is all the more necessary to make sure that it is justified by the experimental observations. Ambard bases it on the fact that the highest concentrations of urea and sodium chlorid which he has ever found in the urine of man are 5.6 per cent. and 2.2 per cent., respectively, and he quotes Bouchardat as authority for the fact that the concentration of glucose may rise to 14.3 per cent. Urea and sodium chlorid have about the same molecular weight but the glucose molecule is nearly three times heavier, so that when the percentage concentrations are expressed as gram mols per liter they become 0.925 for urea, 0.376 for sodium chlorid and 0.789 for glucose. But urea and glucose are nonelectrolytes, while sodium chlorid is largely dissociated, so that the osmotic pressure is greater than the number of molecules per liter in proportion to the degree of dissociation. An 0.736 molar solution of sodium chlorid at 37.5 C. (99.6 F.) is 90 per cent. dissociated, so the osmotic equivalent in gram mols per liter is 0.677. The relative osmotic pressures of 5.6 per cent. urea, 2.2 per cent. sodium chlorid and 14.3 per cent. glucose are thus 0.0925, 0.0677 and 0.0789. These values, however, seem to be too diverse to justify the conclusion that the maximum concentrations of urea, sodium chlorid and glucose are approximately equivalent, or at least the approximation is not sufficiently close to justify speculation based on the supposition that they might be equivalent. And it should be remembered that we have provisionally accepted Ambard's figures as being truly representative. But the 14.3 per cent. concentration of glucose is certainly a very exceptional concentration, much higher than those which we have observed. The greatest glucose concentration

3. Ambard: Physiologie normale et pathologique des reins, Paris, 1914, p. 44.

found by Keith [4] was 7.8 per cent. in experiments on dogs, dehydrated by the intravenous injection of a very large amounts of concentrated glucose solution, conditions which appear to be ideal for producing the highest possible concentrations. Ambard and Papin [1] give concentrations for urea and sodium chlorid which they believed were maximal. They were obtained in dogs under special conditions, an exclusive meat diet for the urea experiments, and the injection of sodium chlorid or the giving of salted water for the sodium chlorid experiments. The osmotic values of the maximal concentrations were always much less for sodium chlorid than for urea. In the six dogs on which maximal concentrations for both urea and chlorid were obtained, the average osmotic pressure of the urea was equivalent to 1.049 molar while the sodium chlorid, after correction for dissociation, had a pressure equivalent to only 0.335. Therefore, either these chlorid concentrations were not maximal or the rule of the uniformity of maximal osmotic pressure values which Ambard believes is followed in man does not hold for dogs. In general, it seems to us that Ambard's results do not at all justify his conclusion.

Adolph [5] has recently reported a large number of observations on himself in which varying but considerable amounts of urea, sodium chlorid and sodium bicarbonate dissolved in very little water were taken. He accepts the idea that there is a maximum concentration for each urinary constituent which the kidney cannot surpass and he concludes that this limit is the same for chlorid, phosphate, urea and bicarbonate, because he considers that the maximal concentrations of all these substances have approximately the same osmotic pressure. But in the first place there is no indication that any absolute maximum is ever reached. In the graphs, the concentrations rise in a curve and do not at any time take the form of a plateau. The urea concentrations which are called maximal vary in five experiments between 0.603 molar (3.65 per cent.) and 0.828 molar (5.02 per cent.). The sodium chlorid concentrations, corrected for ionization, vary from about 0.47 to 0.63 molar. This considerable range of variation is ascribed to a "daily variation concerned with the activities and water level of the entire body," to differences in the work of the kidney preceding the experiments and lastly to a "variation with long stretches of time, as from year to year." But in another experiment, in which he took no water for four days, still another maximum for sodium chlorid was reached, for under these conditions the corrected molar concentration of 0.662 was found. In the second place, the osmotic equality of the maximal concentrations of different substances is not demonstrated. The maximal concentrations

4. Keith: Am. J. Physiol. **63**:394, 1922-1923.
5. Adolph, E. F.: Am. J. Physiol. **65**:419 (Aug.) 1923.

for urea and sodium chlorid are given as 0.678 molar and 0.522 molar (corrected for ionization), although no reasons are given why these particular figures should have been selected. In order to explain why the urea concentration is higher than the sodium chlorid concentration, it is pointed out that the difference between them, which is 0.156 molar, is approximately "the threshhold concentration of sodium chlorid retained in the blood, and against this, of course, the kidneys have to work." But if it is right to add the sodium chlorid concentration of the blood to the chlorid concentration of the urine, why should the urea concentration of the blood not be added to the urea concentration of the urine? The blood urea concentration in normal persons rises to high levels after the administration of such large amounts of urea as were taken by Adolph, and if this had been taken into account there would still have been a discrepancy between the urea and chlorid concentrations. Only one experiment is given for sodium bicarbonate. The corrected molar concentration was 0.617, which is far from equivalent to the highest urea concentration of 0.828 molar. No data are given on phosphate concentrations.

During the last few years we have made a great many serial determinations of the concentration of urea in the urine after abstention from water or fluids for periods of from thirty to forty hours, combined with the repeated administration of considerable amounts of urea. In a few cases these experiments have been repeated many times on the same subject. The concentrations found were not dissimilar from those already reported by Ambard, Adolph and many others. The difference lies not in the facts but in their interpretation. Because it seemed to us quite apparent that the observed concentration might possibly have been surpassed if it had been feasible to continue the abstention from water still longer, we did not feel that we were justified in supposing that we had reached a "maximum" concentration in the sense in which that word is used by Ambard, i. e., "une concentration que l'on ne pourra faire dépasser à l'animal par ancun procédé si artificiel soit-il." This is the only adequate definition, because it is the only one under which maximal concentrations can be regarded as having any theoretic or practical importance. Our failure, and what we regard as the failure of others, to demonstrate the existence of any such maximum concentration does not permit us to conclude that there is no such thing, because in no case have the conditions required for the production of high concentrations been pushed to the ultimate limit. This, however, has now been done. MacKay and MacKay [6] report experiments which were specially performed to cause the greatest possible reduction in water available for excretion by the kidneys, and which were also accom-

6. MacKay, L. L., and MacKay, E. M.: Am. Jour. Physiol. (in press).

panied by an increased rate of urea excretion. These conditions were maintained in some cases until the deprivation of water resulted in the death of the animal. Throughout the entire period the concentration of urea was determined in each collection of urine, and under these most extreme conditions these concentrations should at some time before the death of the animal have reached the "maximum" and thereafter have remained approximately constant. But in none of the sixteen animals used was the rising curve of the urine urea concentration converted into a plateau. Therefore, there is no such thing as a maximum urea concentration in the urine under conditions compatible with life. There is no limitation to the concentration of urea in the urine which is imposed by virtue of any incapacity of the kidney to produce a still more concentrated urine. If it were possible to induce conditions under which there would be even less water and more urea available for excretion, we have no reason to believe that the kidney might not respond with

TABLE 5.—*Average Concentrations as Gram Mols per Liter Corrected for Dissociation, Observed After Decrease in the Rate of Water Excretion Combined with Increase in the Rate of Excretion of the Substances Investigated*

Conditions	Urea	Sodium Chlorid	Sodium Acid Phosphate
Nothing given	0.480	0.250	0.087
Urea, 30 gm.	0.665	.....	.....
Sodium chlorid, 10 gm.	.....	0.505	.....
Sodium acid phosphate, 10 gm.	.....	.....	0.242
Sodium acid phosphate, 10 gm.	0.365	0.208	0.258
Urea, 30 gm.; sodium chlorid, 5 gm.; sodium acid phosphate, 10 gm.	0.596	0.238	0.081

the production of a still higher urine urea concentration. This demonstration that the hypothesis of a maximum concentration which the kidney is unable to surpass is no longer tenable is of fundamental importance, because the general acceptance of this idea has led investigators to ascribe variations in the concentration of urinary constituents to changes in the concentrating capacity of the kidney. But if the full renal capacity is never reached, it is as logical to suppose that these variations may be due to changes in the environment of the kidney rather than to any supposed success or failure of the kidney in performing its work. If the difficulty which is experienced in obtaining very high urinary concentrations is not due to any inability of the kidney itself, it is as reasonable to suppose that it may be the result of the tenacity with which the various regulating mechanisms of the body resist more than a certain amount of deviation from the usual relations which are maintained between the water and the solids in the blood supplied to the kidney.

The hypothesis that "maximal" concentrations of urea, chlorid and phosphate, or rather such unusually high concentrations as may be

attained without too much discomfort to the subject of the experiments, have all approximately the same osmotic pressure may be tested from the experimental data we have given. The average percentage concentrations have been expressed as gram mols per liter, and in the case of the electrolytes, sodium chlorid and sodium acid phosphate, a correction for dissociation has been made from the measurements given by Shaeffer and Clover [7] and by Watkins.[8]

The osmotic equivalents of the highest average concentrations are 0.665 for urea, 0.505 for sodium chlorid and 0.253 for sodium acid phosphate. If we take the highest of all the observations we have 0.800 for urea, 0.674 for sodium chlorid and 0.317 for sodium acid phosphate. It cannot be maintained that there is even an approximate correspondence between these values and, as a matter of fact, when the various consequences which may be inferred from the theory of a limited renal concentrating capacity are no longer entertained, there does not seem to be any good reason to expect that they should be the same. There is, on the contrary, a reason to anticipate that phosphate concentrations might never become osmotically equivalent to high urea concentrations. For, given the same degree of decrease in water excretion, the concentration of that substance will be the highest whose rate of excretion can be most augmented. Now it is not possible to reach any limit to the capacity of the kidney in excreting either urea or phosphate. For when more and more is given the concentration of urea and of phosphate in the blood rises higher and higher, but the kidney continues to respond with an ever increasing rate of excretion, which in the case of urea tends to be directly proportional to the blood urea concentration,[9] and in the case of phosphate also varies in direct relation to the plasma phosphate concentration.[10] If, however, sufficiently concentrated solutions of either urea or phosphate are injected intravenously for a long enough time the muscles will begin to twitch, soon afterward there is a convulsion and the animal dies. Nevertheless, up to the time at which these symptoms appear the kidney is still smoothly and steadily increasing the rate of excretion in correspondence with the mounting blood concentration, so that the limit to the attainment of still higher rates of excretion is imposed, not by the kidney, but by the nervous system. This limit is set at a much lower level for phosphate than for urea. We have been unable to obtain rates of phosphate excretion which exceed 150 mg. sodium acid phosphate hourly per kilogram of body weight without the development of these nervous disturbances, but with urea, rates as high as 1,785 mg. hourly per kilogram of body weight have

7. Shaeffer and Clover: Carnegie Inst. Wash., 1912, No. 170, p. 14.
8. Watkins: Carnegie Inst. Wash., 1915, No. 230, p. 153.
9. Addis, T., and Drury, D. R.: J. Biol. Chem. **55**:105 (Feb.) 1923.
10. Addis, Mayer and Myers: Observations (to be published).

been obtained.[11] There is, therefore, reason to expect that phosphate concentrations in the urine cannot be increased to such a high level of osmotic pressure as is possible for urea, no matter how extreme the conditions may be. The rate of sodium chlorid excretion is limited in much the same way, and possibly for the same reason that the rate of sodium phosphate excretion is restricted.[12] On *a priori* grounds it is therefore improbable that high concentrations of urea, sodium chlorid and sodium acid phosphate will have the same osmotic pressure, for the inequalities in the maximum rates of excretion would have to be counterbalanced by proportional inverse changes in the rates of water excretion. Certainly, under the conditions which have been investigated, even the results of those who have advanced the theory fail to demonstrate any equality between urea and sodium chlorid concentrations, and in the experiments which we have given a pronounced inequality between the molecular ionic concentrations of urea and phosphate has been found.

There is still another hypothesis in which renal incapacity is regarded as the limiting factor in determining urinary concentrations under certain conditions. Chaussin [13] found that "maximal" concentrations of urea could be obtained only when the rate of salt excretion was low, and similarly that "maximal" sodium chlorid concentrations were found only when the conditions were such that the rate of urea excretion was low. He supposes, therefore, that the concentration of one substance is influenced by the concentration of other substances and since he found that the sum of the urea and chlorid concentrations was almost contant, he concludes that the kidney is limited in its capacity to secrete a urine whose total osmotic pressure exceeds a certain level. "La charge de l'organisime en sel diminue la capacité du rein vis-à-vis de l'élimination de l'urée et reciproquement." Our own observations might be regarded as supporting this theory, for the highest concentrations of urea, chlorid and phosphate were obtained only when each substance was given alone. On the other hand, we failed to reach any limit to the total concentrating capacity of the kidney, for the total gram molar concentration, corrected for ionization, increased from 0.665 with decrease in water excretion, to 0.769 when urea, chlorid and phosphate were taken together. However, if the kidney is really incapable of excreting high concentrations of urea and chlorid at the same time, its failure should become very manifest when the conditions are more rigorous than was possible in Chaussin's or in our experiments. We accordingly gave urea and sodium chlorid by stomach tube to rabbits deprived of water. The results are given in Table 6.

11. Drury, D. R.: J. Biol. Chem. **55**:113 (Feb.) 1923.
12. Munzer: Arch. f. exper. Path. u. Pharmakol. **41**:74, 1898.
13. Chaussin: J. de Physiol. et de path. gén. **18**:895, 1919-1920.

It is evident from these results that the kidney is able to secrete urine in which the concentration of both urea and chlorid is high. But when the rate of excretion of urea alone is increased, the increased rate of water excretion which is associated with that increase will, of course, lower the concentration of chlorids, just as a marked increase in the rate

TABLE 6.—*Results of Administration of Urea and Sodium Chlorid in Four Rabbits*

Conditions	Time of Urine Collections, Number of Hours Without Water	Volume, C.c. per Kilogram of Body Weight Hourly	Urea Percentage, Gm. per 100 C.c.	Sodium Chlorid Percentage, Gm. per 100 C.c.
Rabbit 1. Weight at One Hundred and Twentieth Hour, 2,000 Gm.				
No water or food for 120 hours....	?-120th	....	5.22	0.07
	120-123d	12.7	3.00	0.41
	123-126th	7.7	3.25	0.25
	126-129th	3.3	4.92	0.26
	129-132d	2.3	5.74	0.33
	132-135th	1.8	5.75	0.49
	135-147th	1.5	5.18	0.93
At 147th hour 5 gm. urea and 1 gm. sodium chlorid in 20 c.c. water	147-151st	5.8	3.94	0.76
	151-155th	3.4	5.42	0.82
	155-159th	2.9	5.92	1.00
	159-171st	1.2	6.12	0.65
At 171st hour 1 gm. sodium chlorid in 20 c.c. water	171-176th	0.5	2.70	0.82
	176-183d	0.7	4.85	0.96
Died soon after 183d hour.....	183-?	...	4.06	0.94
Rabbit 2. Weight at Forty-Eighth Hour, 2,000 Gm.				
No water or food for 48 hours.....	?- 48th	....	3.99	0.01
At 48th hour 10 gm. urea in 20 c.c. water	48- 55th	10.0	4.29	0.09
	55- 70th	2.3	6.60	0.00
At 75th hour 5 gm. urea and 1 gm. sodium chlorid in 20 c.c. water	70- 79th	4.4	5.59	0.51
	79- 90th	1.8	6.94	0.51
At 90th hour 5 gm. urea and 1 gm. sodium chlorid in 20 c.c. water	90-111th	3.6	5.28	0.66
Died soon after 111th hour.....	111- ?			
Rabbit 3. Weight at Forty-Eighth Hour, 2,350 Gm.				
No water or food for 48 hours.....	? - 48th	....	2.91	0.18
At 48th hour 11.75 gm. urea in 23.5 c.c. water	48 - 55th	9.7	4.05	0.23
	55 - 70th	8.5	7.20	0.08
At 70th hour 5.88 gm. urea and 1.25 gm. sodium chlorid in 23.5 c.c. water	70 - 79th	4.5	6.13	0.52
	79 - 90th	1.0	6.78	1.00
At 90th hour 5.88 gm. urea and 1.25 gm. sodium chlorid in 23 5 c.c. water	90 -111th	3.6	5.34	0.80
	111 -123d	1.1	6.51	0.29
	123 -134.5	1.1	6.36	0.26
	134.5-148.5	0.8	6.74	0.01
At 148.5 hour 5.88 gm. urea and 1.25 gm. sodium chlorid in 23.5 c.c. water	148.5-152.5	0.9	4.42	0.01
Died at 152.5 hour				
Rabbit 4. Weight at Fifty-Fifth Hour, 1,900 Gm.				
No food or water for 55 hours.....	?- 55th	....	4.04	0.00
At 55th hour 9.9 gm. urea in 19 c.c. water	55- 70th	5.3	5.61	0.03
At 70th hour 4.99 gm. urea and 0.99 gm. sodium chlorid and 0.99 gm. acid sodium phosphate in 19 c.c. water	70- 79th	4.7	6.27	0.21
	79- 90th	1.5	6.81	0.01
At 90th hour 4.99 gm. urea and 0.99 gm. sodium chlorid and 0.99 gm. acid sodium phosphate in 19 c.c. water	90-103d	2.8	5.07	0.69
	103-115th	2.3	5.10	0.94
	115-126.5	0.2	2.69	0.01
Died soon after 126th hour				

Conditions	Time of Urine Collections, Number of Hours Without Water	Volume, C.c. per Kilogram of Body Weight Hourly	Urea Percentage, Gm. per 100 C.c.	Sodium Chlorid Percentage, Gm. per 100 C.c.	Acid Sodium Phosphate Percentage, Gm. per 100 C.c.
Rabbit 4. Weight at Fifty-Fifth Hour, 1,900 Gm.					
No food or water for 55 hours.....	?- 55th	....	4.04	0.00	1.56
At 55th hour 9.9 gm. urea in 19 c.c. water	55- 70th	5.3	5.61	0.03	0.31
At 70th hour 4.99 gm. urea and 0.99 gm. sodium chlorid and 0.99 gm. acid sodium phosphate in 19 c.c. water	70- 79th	4.7	6.27	0.21	0.83
	79- 90th	1.5	6.81	0.01	1.61
At 90th hour 4.99 gm. urea and 0.99 gm. sodium chlorid and 0.99 gm. acid sodium phosphate in 19 c.c. water	90-103d	2.8	5.07	0.69	0.76
	103-115th	2.3	5.10	0.94	0.82
	115-126.5	0.2	2.69	0.01	0.46
Died soon after 126th hour					

of chlorid excretion will, as a rule, lead to a decrease in the urea concentration. Chaussin's findings may be explained on these grounds, and it is unnecessary to assume that the kidney is incapable of producing high concentrations of both simultaneously. Whether this is true for all urinary constituents it is impossible to say. There are certain effects on chlorid excretion produced by the administration of phosphate for which we have no explanation, but certainly the suggestion that they are due to renal incapacity seems to us more improbable than many others which might be given.

TABLE 7.—*Association Between Rate of Water and Rate of Substance Excretion*

Conditions	Water, C.c. in 12 Hours	Urea, Gm. in 12 Hours	Urea + Chlorid + Phosphate, Gm. in 12 Hours
Substance = Urea			
Phosphate, 10 gm.	227	4.89	10.40
Nothing	243	5.40	.....
Urea, 30 gm.	596	23.20	.....
Urea, 30 gm.; sodium chlorid, 5 gm.; sodium acid phosphate, 5 gm.	618	22.02	30.00
Substance = Sodium Chlorid			
Phosphate, 10 gm.	227	1.51	10.40
Nothing	248	1.78	.....
Sodium chlorid, 10 gm.	364	6.04	.....
Urea, 30 gm.; sodium chlorid, 5 gm.; sodium acid phosphate, 5 gm	618	4.64	30.00
Substance = Sodium Acid Phosphate			
Phosphate, 10 gm.	227	4.00	10.40
Nothing	243	1.28	.....
Phosphate, 10 gm.	222	3.75	.....
Urea, 30 gm.; sodium chlorid, 5 gm.; sodium acid phosphate, 5 gm.	618	3.34	30.00

We have shown that the concentration of any substance in the urine is limited by the impossibility of increasing the rate of excretion of the substance beyond a certain point, without the production of symptoms which terminate in the death of the animal. If this were the only limitation the range of observed concentrations would be much wider than it is. But there is, of course, another factor to be considered, the rate of water excretion. Theoretically, the optimum conditions for the production of high concentrations consist in as great as possible an increase in the rate of excretion of the solid substance with at the same time, the maintenance of the rate of water excretion at a minimum value. But we found that whenever we succeeded in producing a marked increase in substance excretion we failed to keep the urine volume low. In Table 7, the average rates of water and substance excretion are given for all conditions.

If the water excretion when nothing was given is compared with the water excretion when each substance was given alone, it will be noted that the greatest increase occurs with urea, the substance whose rate of excretion was most increased. With sodium chlorid a moderate increase in rate of excretion is associated with a moderate rise in urine volume. With phosphate there was a slight decrease in volume but there was little increase in the rate of excretion, and there was at the same time some diminution in the rate of urea and chlorid excretion. There is, therefore, evidence of a relation between the excretion of solids and of water, and it seems likely that it is the total solid excretion which is related to the volume of urine, for in the experiments in which urea, chlorid and phosphate were all determined, the volume of urine varies in almost direct proportion to the sum of these three substances. It is evidently this tendency toward a parallel increase in water as well as in solid excretion, which is the real cause of the diffculty in obtaining very high urinary concentrations. It has been assumed that the water excretion is increased because the kidney is unable to excrete a substance at more than a certain maximum concentration. But since we have failed to find any evidence of any renal incapacity in concentrating it seems as reasonable to suppose that, under conditions in which the rate of excretion of any substance is markedly increased, the amount of water available for excretion may also be increased. The physical conditions within the body may be such that a pronounced increase in the concentration of any substance in the blood, which is the necessary precursor of any great increase in its rate of excretion, is inevitably accompanied by an increase in the concentration of free water in the blood. The absence of a very high concentration of any solid in the urine would then be only what was to be expected if the kidneys were efficient in eliminating together both solid and water, when their concentrations in the blood became greater than usual.

A very extreme instance of the relation between urea and water excretion is provided by experiments carried out in this laboratory by Drury.[11] By means of a Woodyatt pump, increasing amounts and concentrations of urea were injected intravenously into a rabbit anesthetized by chlorbutanol. A 0.9 per cent. solution of sodium chlorid without urea was first injected at the rate of 27.3 c.c. hourly per kilogram of body weight, and then on different days 2 per cent. urea at 27.3 c.c. hourly per kilogram of body weight, 2.5 per cent. urea at 44.4 c.c. hourly per kilogram of body weight and finally 5 per cent. urea at the rate of 78.3 c.c. hourly per kilogram of body weight. The concentrations of urea in the urine and in the blood and the rates of urea and water excretion are given in Table 8.

As the concentration of urea in the solution which was injected is increased to 5 per cent., there is no corresponding increase in the concen-

tration of urea in the urine. Since we have often found concentrations of over 7 per cent. in the urine of rabbits we cannot suppose that the kidneys of this animal were unable to produce a urine containing 5 per cent. urea. Nor is the relatively low urine urea concentration due to any falling off in the rate of urea excretion when the 5 per cent. urea solution was injected, since the increase in the rate was at that time proportionally greater than the increase in blood urea concentration. The 1.52 per cent. concentration observed when 5 per cent. urea was given was clearly due to an increase in water excretion, which was even larger than the increase in urea excretion. When a rate of water excretion of 120.5 c.c. hourly per kilogram of body weight is obtained under conditions in which only 78.3 hourly per kilogram of body weight is injected, it would seem reasonable to look for some cause outside of the

TABLE 8.—*The Effect of the Injection of Increasing Concentrations of Urea on the Concentration of Urea in the Urine When the Quantity of Urea Given Is Large Enough to Greatly Increase the Concentration of Urea in the Blood*

Solution Injected, Concentration of Urea	Urine Concentration of Urea, Gm. per 100 C.c	Blood Concentration of Urea, Gm. per 100 C.c	Urine Rate of Urea Excretion, Gm. per Kg. of Body Weight Hourly	Urine Rate of Water Excretion, C.c. per Kg. of Body Weight Hourly
0.0%	0.63	0.03	0.044	7.3
0.0%	1.14	0.03	0.041	3.6
2.0%	1.01	0.15	0.242	24.1
2.0%	1.71	0.17	0.210	12.3
2.0%	1.39	0.17	0.246	17.7
2.5%	2.16	0.38	0.669	30.9
2.5%	1.79	0.48	0.941	52.7
5.0%	1.52	0.76	1.827	120.5

kidney altogether, and the most likely explanation is, of course, that the injection of large amounts of a concentrated urea solution led to an increase in the concentration of free water in the blood. This particular experiment is cited because it clearly shows the improbability of theories based on the assumption of renal incapacity. For this rabbit excreted during the last period amounts of urea and water equivalent to an excretion by a man weighing 70 kg. of over 3,000 gm. of urea and of over 200,000 c.c. of water every twenty-four hours. In view of this enormous capacity for work in elimination, very definite evidence should be required before the hypothesis of a renal incapacity to concentrate is seriously considered.

As a general rule, the introduction of speculations in regard to the specialized function of the several components of the glomerulotubular element produces only a greater confusion in the consideration of the concentrating capacity of the kidney. But in the case of urea we have very definite anatomic evidence of its existence in higher concentration within the cells of the proximal convoluted tubule than elsewhere in the

kidney or in the other tissues of the body. This was first shown by Leschke,[14] with the mercury method, and then by Oliver,[15] with the xanthydrol method. Oliver's results have since been fully confirmed by several observers.[16] As Oliver has pointed out, any hypothesis to account for this high urea concentration within these particular cells other than that they are actively engaged in excreting urea from the blood, entails highly improbable assumptions, and since the recent physiologic work of Marshall and Crane [17] leads to the same conclusion, it would seem that the theory that the excretion of urea is accomplished mainly by the proximal convoluted tubule is more firmly based on observed fact than are hypotheses in regard to the mode of excretion of other urinary constituents. But, though that part of the tubule may be the only place where a true secretion occurs, it seems likely that some urea passes with water through the glomerulus, and unless we assume an impermeability of the glomerulus for urea, or a special secretory activity of the glomerular epithelium for urea, for neither of which is there any evidence, we must suppose that the concentration of the urea which enters Bowman's capsule is identical with that which exists in the blood. Now, under ordinary conditions the urea which passes through the glomerulus may be insignificant in amount in comparison with the urea which passes in high concentration through the long proximal convoluted tubule, but, under the very extreme conditions of the experiment we have just cited, this glomerular fraction might become important. When it is remembered that a relatively enormous amount of fluid was being injected, and that life was only maintained by virtue of the ability of the kidney to excrete water at a very great rate, it seems unlikely that any appreciable amount of water which had passed into the lumen of the tubules from the glomeruli would be reabsorbed into the body again by the tubule cells. If this is granted, the volume of urine then becomes a measure of the greatest possible amount of water which could have been excreted through the glomeruli, the actual amount being less in proportion to the amount of water which may have passed through the tubule cells. And since we know the concentration of urea in the blood during each period of urine collection we can determine the amount of urea which may have been excreted by the glomeruli during a given time, and by substracting this value from the total urea excretion we obtain the maximal possible glomerular rate of urea excretion and the minimal possible tubular rate of urea excretion. These values have been calculated from the data in Table 8 and are given in Table 9.

14. Leschke: Ztschr. f. klin. Med. **81**:14, 1915.
15. Oliver, Jean: J. Exper. Med. **33**:177 (Feb.) 1921.
16. Stubel: Anat. Anzeig. **54**:236, 1921. Piras: Arch. de Fisiol. **20**:237, 1922. Walter: Arch. f. d. ges. Physiol. **198**:267, 1923.
17. Marshall, E. K., Jr., and Crane, U. U.: Am. J. Physiol. **62**:330 (Oct.) 1922.

The results indicate that only a practically negligible fraction of the total urea excretion can pass through the glomeruli, even with large urine volumes, so long as the blood urea concentration is not markedly increased. But when the blood urea concentration rises to very high levels and at the same time the volume of glomerular filtrate is greatly augmented, the proportion of the total urea which is excreted by the glomeruli certainly becomes larger and larger until it might conceivably contribute almost half of all the urea eliminated under the unusual conditions of the last experiment. Now it has been demonstrated that under constant conditions the rate at which the same amount of renal tissue will excrete urea varies in direct proportion to the concentration of urea in the blood, so that the ratio: $\frac{\text{rate of urea excretion}}{\text{blood urea concentration}}$ is a constant.[2] These urea injection experiments were carried through in order to determine whether the kidney would be capable of excreting the very large amounts of urea which would be required if the relationship were

TABLE 9.—*Calculated Rates of Glomerular and Tubular Urea Excretion per Kilogram of Body Weight*

Total Rate, Gm. per Hour	Glomerular Rate, Gm. per Hour	Tubular Rate, Gm. per Hour	Blood Urea Concentration, Gm. per 100 C.c.	Ratio: $\frac{\text{Total Rate}}{\text{Blood Conc.}}$	Ratio: $\frac{\text{Tubular Rate}}{\text{Blood Conc.}}$
0.044	0.002	0.042	0.027	1.63	1.56
0.041	0.001	0.040	0.027	1.53	1.50
0.242	0.035	0.207	0.149	1.63	1.39
0.210	0.020	0.190	0.166	1.27	1.14
0.246	0.031	0.215	0.174	1.41	1.25
0.669	0.117	0.552	0.377	1.77	1.46
0.941	0.254	0.687	0.482	1.95	1.43
1.827	0.909	0.918	0.755	2.42	1.22

to hold when the blood urea concentration was increased from about 25 to over 750 mg. per hundred cubic centimeters. The results showed that the kidney did not fail even under this very great and unusual load, for the value of the ratio did not decrease, as would have been the case had the rate of urea excretion failed to increase in proportion to the increase in blood urea concentration. It will be noted that the magnitude of the ratio, which is given in the fifth column in Table 9, not only does not decrease, but that on the contrary, it becomes larger. This increase in the ratio in the last three experiments, when the blood urea concentration had risen to over 550 mg. per hundred cubic centimeters, finds an adequate explanation in the actual inconstancy of the conditions which were such as to lead to an increasingly important increment of urea from the glomeruli, a source which does not follow any direct proportional relation to the blood urea concentration, since it varies also with changes in the volume of the glomerular filtrate. Although such considerations involve an element of speculation, they are supported by the fact that the ratios became approximately constant when the possible inconstancy in

the conditions is eliminated by calculating them in relation to the tubular rates instead of to the total rates of urea excretion [18] (Table 9).

The fact that there is no evidence that the kidney puts any restriction on the height to which the concentration of urea in the urine may rise gives us no right to assume that it may not limit the depth to which urine urea concentrations may fall. Knowing that urea is excreted by the proximal tubule in a higher concentration than that of the blood, and assuming that the glomerular filtrate has the same concentration as exists in the blood, it is clear that the lowest concentration of urea which can occur in the urine must be higher than the concentration of urea in the blood. The kidney would thus impose a limitation on the degree to which urea may be diluted in the urine, a limitation which would be a result of the mechanism adopted for the excretion of urea. But it may be that the elimination of urea is in reality more complicated than we suppose. There might, for instance, be a reabsorption of urea from the urine by the kidney, or at some part of its structure the kidney might be capable of excreting pure water without urea. If either of these two latter suppositions were correct, we might be able to obtain urine with a concentration of urea which was less than that present in the blood during the period in which it was produced. We have endeavored to induce the secretion of such a urine by injecting large amounts of salt solution or of water into rabbits, or by combining these injections with the administration of pituitary extract which decreases the rate of urea excretion,[19] but we never succeeded in reducing the concentration of urea in the urine below that of the blood. During the last ten years we have also made many synchronous observations of the urea concentration in the blood and urine of patients with uremia, but have always found a higher concentration in the urine than in the blood. While these negative results do not exclude the possibility of pure water excretion or of urea reabsorption, they at least indicate that there is no need to complicate a matter which is already sufficiently intricate by hypotheses which are unnecessary and on other grounds inherently improbable.

COMMENT

To anyone who has read this paper in the hope that some new facts might be presented which would at least make the subject more concrete,

18. Addis and Drury (J. Biol. Chem. **55**:639, 1923), after many experiments on themselves, came to the conclusion that the rate of water excretion had no appreciable direct effect on the rate of urea excretion, but in this conclusion they overlooked the significance of the experiments we have discussed. In the exceptional circumstances of a very marked increase in both blood urea concentration and urine volume, any further increase in the rate of water excretion will cause an increase in the rate of urea excretion, because it will add an appreciable amount of urea through increased glomerular filtration.

19. Addis, Thomas, Barnett, and Shevky: Am. J. Physiol. **46**:52 (May) 1918.

it will be apparent that the really fundamental questions have not even been approached. Such questions, however, cannot be formulated now, because the elementary facts of observation on which they will be based require methods for their determination which are not yet in existence. A first step, for example, is an exact knowledge of the concentrations which occur within the cells of the various sections of the glomerulotubular apparatus, but these concentrations will remain unknown until a quantitative histochemical method has been elaborated. But it will be admitted that, in our present state of ignorance, it is of some importance that no preliminary hypothesis should be accepted without thorough criticism, particularly if that hypothesis is used as a foundation for still other and wider hypotheses relating to renal physiology or if it is utilized in explaining functional deviations observed in patients. Ambard's views on this subject have been accepted and widely used, but we have tried to show that the facts from which they are derived can be coordinated under another hypothesis than his, one which is simpler and more in accordance with probability, and we have adduced still other facts which we believe make his position untenable. We maintain that at least the superficial aspects of all that we know about changes in the concentration of urea, chlorids and phosphates in the urine can be explained on the assumption that they duplicate parallel changes in the relation between the concentration of these solids in the blood and the concentration of the free water of the blood. We need not assume any incapacity on the part of the kidney itself in order to explain the difficulty experienced in obtaining very high concentrations in the urine. The experiments we have given do not reveal any such incapacity even under the most extreme conditions, and in any case it is an unnecessary assumption. We start with the knowledge that, by definition, the concentration in the urine depends on the relation between the rate of excretion of the substance and the rate of water excretion. Observation shows that when the rate of excretion of the substance is much increased there is also an increase in the rate of water excretion, and that this is the reason why the concentration of a substance in the urine never reaches a very high level. It is also a fact of observation that any marked increase in the rate of urea or of phosphate excretion is invariably accompanied by a marked and proportional increase in the concentration of urea and phosphate in the blood. In order to explain the increased water excretion which accompanies high rates of excretion and which keeps the concentration of urine from rising as high as it otherwise might, we assume that any pronounced increase in the concentration of the substance in the blood is associated with a parallel increase in the concentration in the blood of the free water which is available for excretion by the kidney. This assumption is not improbable on the basis of general physical analogy, but for the present it must remain an

assumption because no method has as yet been devised by means of which the concentration of free water in the blood can be measured. In a paper to be published, observations dealing with the cause of the low concentrations of urea found in the urine of patients in the terminal stages of Bright's disease will be given.

CONCLUSIONS

1. Experiments on normal human subjects are reported, in which the conditions were adjusted with a view to the production of urine which would contain the highest possible concentration of urea, chlorids and phosphates. It was found that the highest concentration of each of these substances was attained when it was given in considerable amount and in high concentration to subjects whose fluid intake had been restricted.

2. Experiments on rabbits deprived of water and given large amounts of a mixture of urea and sodium chlorid showed that it was possible to obtain urine in which there was a high concentration of both of these substances. No evidence was found which would suggest that the height to which the osmotic pressure of the urine might rise is restricted by virture of any limitation in renal capacity.

3. The widely accepted hypothesis that the kidney is unable to excrete urea, chlorid or phosphates at more than certain maximum concentrations is shown to be untenable.

4. The assumption that concentrations of urea, chlorid and phosphate, which approach the highest levels found in the urine, have approximately the same osmotic pressure is shown to be contrary to observed facts.

5. It is suggested that changes in the concentration of urea, chlorids and phosphates in the urine are produced by changes in the relation between the concentration of these substances in the blood and the concentration of free water in the blood. It is difficult to obtain very high concentrations of the urine because a marked increase in the concentration of urea, chlorid or phosphate in the blood is associated with a concomitant increase in the concentration of free water in the blood.

THE PRESENT STATUS OF THE QUESTION OF THE LENGTH OF LIFE OF THE UNAGGLUTINABLE TRANSFUSED RED BLOOD CORPUSCLE

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Isaacs,[1] in a recent publication from the Harvard Medical School and allied hospitals, has presented data which apparently establish a new and much needed means of identifying young red blood corpuscles. These cells which appear in the circulation after transfusion, can be distinguished by their content of one or more of four kinds of granules, of which the most important appears to be a comparatively large, non-staining refractile body. Isaacs has also quantitatively correlated the occurrence of granular and reticulated corpuscles to the presence of cells nonagglutinable with serum, containing isoagglutinins against the majority of the native cells of the circulation in question. So far as Isaacs' work concerns itself with the significance of the cells containing the refractile granule, I predict that it may prove a valuable contribution, but with respect to his conclusions concerning the tenure of life of the unagglutinable transfused corpuscle I do not agree.

Isaacs has made a modification of a technic which I introduced to follow the life of the Group IV (Moss) transfused corpuscle in the circulation of a recipient of another group, which is accomplished by agglutinating the recipient's corpuscles with Group IV serum and subsequently counting the remaining unagglutinated corpuscles in a hematocytometer. By means of the modification Isaacs considers that he has shown that the transfused unagglutinable corpuscles disappear and are replaced by unagglutinable young cells put into the circulation by the host. In the two published cases this replacement is complete in four days.

I consider that Isaacs has not proved his point for the following reason: The data in his two reported cases are in gross disagreement with my data in which forty-two cases were reported,[2] and with that

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1. Isaacs, Raphael: Properties of Young Erythrocytes in Relation to Agglutination and Their Behavior in Hemorrhage and Transfusions, Arch. Int. Med. **33**:193 (Feb.) 1924.

2. Ashby, Winifred: The Determination of the Length of Life of Transfused Blood Corpuscles in Man, J. Exper. Med. **29**:267-281 (March) 1919; Some Data on the Range of Life of Transfused Blood-Corpuscles in Persons Without Idiopathic Blood Diseases, M. Clin. N. Amer. **3**:783-799 (Nov.) 1919-1920; Study of Transfused Blood. I. The Periodicity of Eliminative Activity Shown by the Organism, J. Exper. Med. **34**:127-146 (Aug.) 1921; Study of Transfused Blood. II. Blood Destruction in Pernicious Anemia, J. Exper. Med. **34**:147-166 (Aug.) 1921.

of Wearn,[3] who also worked with this technic, both as to the number of unagglutinable cells found after a transfusion and as to the length of time that they are found in the circulation. The fact that like group transfusions do not produce an increase in the unagglutinable corpuscles offers a serious objection to Isaacs' conclusion that transfused Group IV corpuscles are replaced within four days by unagglutinable native cells. Moreover, his technic is open to certain objections.

The most serious obstacle to Isaacs' conclusion, that after four days the unagglutinable corpuscles found in the circulation are the young native cells which have replaced the transfused cells, seems to me to be the fact that it has already been shown by my data and also by that of Wearn that the like group transfusion does not produce this increase in unagglutinable corpuscles. As my data show that with the Group IV transfusion there is a sustained increase in unagglutinable corpuscles which is in proportion to the amount of blood transfused and the probable blood volume of the recipient, in order to meet this objection, we would have to assume not only that the Group IV transfusion causes a prolonged stimulation of the bone marrow while the like group transfusion does not, but also that the stimulation was so adjusted that the number of new cells, produced as a result of the stimulation, consistently maintained the same relationship in all patients to the amount of blood originally transfused, and the blood volume of the recipient. Although this is theoretically possible, it is highly improbable, especially in view of the fact that I have found no difference in the subsequent effect of the two types of transfusion on the rise of the total red count.

In my own reported work are charted thirty-seven instances, and in the data of Wearn there are three, in which like group blood was transfused while the unagglutinable corpuscles were being studied, and practically no change in the count of unagglutinable corpuscles was found to take place following the like group transfusion. In Table 1, I am giving in detail the change in the count of unagglutinable corpuscles following a series of Group IV and like group transfusions in a single case (Case 57, already reported). The case was that of a man in Group II with pernicious anemia, which I have chosen because it offers an example of all the possible combinations: (1) an initial Group IV transfusion; (2) a Group II transfusion following a Group IV transfusion; (3) a Group II transfusion after a period of rest; (4) a Group IV transfusion following a Group II transfusion, and (5) a Group IV transfusion following a group IV transfusion. These combinations would seem to answer fully any questions that might arise as to whether the failure of unagglutinable corpuscles to appear in increased

3. Wearn, J. T.; Warren, Sylvia; and Ames, Olivia: The Length of Life of Transfused Erythrocytes in Patients with Primary and Secondary Anemia, Arch. Int. Med. **29**:527-538 (April) 1922.

numbers after a like group transfusion following a Group IV transfusion might be due to a change in the bone marrow activity resulting from stimulation. It will be seen that after each Group IV transfusion there is approximately the same marked rise, while after the like group transfusions the counts of unagglutinable corpuscles practically do not change (Table 1). In no instance, either in the studies of Wearn or in my reported forty-two cases, has there failed to occur a marked increase in the count of unagglutinable corpuscles following a trans-

TABLE 1.— *The Failure of the Count of Unagglutinable Corpuscles to Increase After a Transfusion of Blood of the Same Type as That of the Recipient, and Its Invariable Rise After a Tranfusion of Unagglutinable Blood*

(Case 57, Man, Group II)

Date	Count of Unagglutinable Corpuscles for Each Cubic Millimeter	Days After Transfusion	Difference Between Counts of Unagglutinable Cells Before and After Transfusion
5/20/19	0.011	...	.....
5/20/19 500 c.c. of Group IV citrated blood transfused			
5/21/19	0.479	1	+0.468
5/26/19	0.488	...	.....
5/26/19 500 c.c. of Group II citrated blood transfused			
5/29/19	0.466	3	—0.022
6/ 3/19	0.375	...	.....
6/ 3/19 475 c.c. of Group II citrated blood transfused			
6/ 4/19	0.354	1	—0.021
6/ 9/19	0.383	6	+0.018
6/16/19	0.388	...	.....
6/16/19 500 c.c. of Group II citrated blood transfused			
6/17/19	0.387	1	—0.001
8/ 4/19	0.208	...	.....
8/ 5/19 500 c.c. of Group II citrated blood transfused			
8/ 6/19	0.181	1	—0.027
8/11/19	0.144	6	—0.064
8/11/19	0.144	...	.....
8/11/19 500 c.c. of Group IV citrated blood transfused			
8/11/19	0.571	0	+0.427
8/13/19	0.500	3	+0.366
8/18/19	0.554	7	+0.410
8/18/19	0.554	...	.....
8/18/19 500 c.c. of Group IV citrated blood transfused			
8/21/19	0.940	3	+0.386
8/22/19	0.940	...	.....
8/22/19 500 c.c. of Group II citrated blood transfused			
8/26/19	0.840	4	—0.100

fusion with unagglutinable corpuscles, and in no instance, in which a like transfusion has been given, has there been found to be any change in the unagglutinable corpuscles, except of a slight degree such as might be accounted for by legitimate technical error, or, when there is a high count resulting from a previous Group IV transfusion, by probable change in blood volume. In my opinion this alone disproves Isaacs' contention.

Isaacs introduces an error into his calculations by computing the transfused corpuscles in terms of percentages of the total red count. Within a period of a few hours, provided that during that time no transfusion had been given, it would be fairly safe to assume that no change

in the absolute number of corpuscles in the circulation had taken place, and that any apparent change was due to blood volume fluctuations. Under these circumstances it would not only be permissible, but preferable, to base the percentage of the unagglutinable count on the total red count, as that would cancel the error due to any blood volume change. Over an extended time, however, such as these studies involve, it must be assumed that the native cells, which constitute the greater part of the total red count, are capable both of entering and of being removed from the circulation independently of the unagglutinable corpuscles. In Isaacs' second case, for instance, by the fourth day after transfusion, the total red count had increased from 1,000,000 to 2,400,000. By basing the change in the number of unagglutinable corpuscles on the total red count, he has introduced an error of approximately 140 per cent., which, of course, is too great to be permissible. The total red count and the count of the unagglutinable corpuscles, whether these are regarded as transfused cells or as young native cells, have no necessary relationship to one another, and unless within the small changes that may fairly be attributed to blood volume fluctuations, the total red count remains constant, any estimate of a change in the transfused corpuscles that is based on a percentage of the total red count is incorrect.

I have taken the liberty of transposing the percentages given in the two cases reported by Isaacs to counts for each cubic millimeter of whole blood, and have compared the results obtained by him with my published data, and that of Wearn, Warren and Ames. These are included in Table 2, which compares the count of the unagglutinable corpuscles found in the circulation after transfusion with the amount of Group IV blood transfused. In Isaacs' cases and in all of mine that were not emergency transfusions, is given the increase in the count of unagglutinable corpuscles resulting from transfusion over the initial count of unagglutinable corpuscles (Table 1). The initial count of unagglutinable corpuscles is the count of the few corpuscles which remain free after a pure blood of an agglutinable type has been treated with an excess of agglutinating serum. The number remaining free depends partly on the potency of the serum, and partly on a quality of the corpuscles which makes them more or less agglutinable. As corpuscles deteriorate in vitro, for instance, their agglutinability decreases and the number of free corpuscles found after agglutination becomes greater; Group III corpuscles have been reported to be, in some instances, weakly agglutinable. In any one subject I have found this initial degree of unagglutinability to be rather constant, but different subjects show considerable variation, as may be seen from Table 2. In cases of obstructive jaundice the agglutinability of the patient's corpuscles has been reduced; the clumps formed have been small and there has been

TABLE 2.—*A Comparison of the Degree of Increase in the Count of Unagglutinable Corpuscles in Relation to the Amount of Unagglutinable Blood Transfused, as Obtained by Isaacs, with That Reported by Wearn, Warren and Ames, and by Ashby*

	Diagnosis	Volume Transfused, C.c.	Count of Unagglutinable Corpuscles, Millions			Days After Transfusion
			Before Transfusion	After Transfusion	Difference	
Cases reported by Isaacs [1]						
	Secondary anemia	400	a. 0.103	0.236	0.131	1
			b. 0.103	0.273	0.158	2
			c. 0.105	0.209	0.104	4
			Av. 0.105			
	Primary anemia	600	a. 0.087	0.170	0.105	1
			b. 0.053	0.096	0.027	4
			Av. 0.069	0.055	—0.014	6
Cases reported by Wearn, Warren and Ames						
1	Primary anemia	575	.....	0.536	.....	5
2	Primary anemia	400	.....	0.465	.....	2
3	Primary anemia	250	.....	0.238	.....	3
5	Chronic nephritis	624	.....	0.624	.....	3
6	Acute nephritis	550	.....	0.275	.....	3
7	Nephritis	250	0.046	0.261	0.215	10
8	Nephritis (boy)	250	.....	0.451	.....	2
Cases reported by Ashby						
6	Hemorrhage	1,200	0.088	1.412	1.324	2
7	Secondary anemia	500	0.011	0.391	0.380	6
8	Exophthalmic goiter	500	0.012	0.495	0.483	5
90	Malaria	500	0.027	0.451	0.424	1
199	Hemorrhage	500	0.033	0.500	0.477	7
115	Secondary anemia	500	0.067	0.692	0.645	3
G	Anemia (infant)[1]	250	0.054	1.190	1.146	5
81	Aplastic anemia	500	0.025	0.478	0.448	0
57	Primary anemia	500	0.011	0.488	0.477	6
43	Primary anemia (163 pounds)	400	0.012	0.260	0.248	4
54	Primary anemia	450	0.022	0.464	0.442	3
26	Primary anemia	460	0.023	0.369	0.346	6
71	Primary anemia	490	0.025	0.554	0.529	3
89	Primary anemia	500	0.027	0.628	0.601	3
103	Primary anemia	500	0.015	0.612	0.597	4
45	Primary anemia	500	0.045	0.570	0.525	4
91	Primary anemia	350	0.053	0.468	0.415	2
59	Primary anemia (short, fat)	400	0.069	0.713	0.644	6
61	Primary anemia	500	0.061	0.641	0.580	3
23	Primary anemia	500	0.080	0.642	0.562	5
23	Two years later	400	0.067	0.441	0.374	4
73	Primary anemia	500	0.086	0.454	0.368	5
80	Primary anemia	500	0.115	0.560	0.445	4
60	Primary anemia	500	0.170	0.693	0.523	4
68	Primary anemia (82 pounds)	500	0.229	1.196	0.967	3
21	Obstructive jaundice	500	0.154	0.670	0.526	4
55	Obstructive jaundice	500	0.220	0.720	0.500	3
47	Hyperthyroid	500	.....	0.684	.....	21
48	Cancer of breast	500	.....	0.565	.....	25
76	Secondary anemia	500	.....	0.532	.....	8
25	Obstructive jaundice	500	.....	0.623	.....	10
38	Hemorrhage	500	.....	0.759	.....	5
50	Hemorrhage	500	.....	0.393	.....	10
76	Secondary anemia	500	.....	0.532	.....	8
92	Colloid goiter (215 pounds)	500	.....	.....	.....	15
92	Colloid goiter (215 pounds)	500	.....	0.622	.....	6
96	Malignant pelvic tumor	500	.....	0.470	.....	10
63	Hemorrhage	500	.....	0.626	.....	9
83	Carcinoma of kidney	500	.....	0.512	.....	4
95	Hemorrhage	500	.....	0.422	.....	4
107	Pernicious anemia	500	.....	0.594	.....	5
116	Purpura	500	.....	0.555	.....	5
C	Carcinoma	500	.....	0.698	.....	5

a comparatively large number of free corpuscles before transfusion with the Group IV blood, as may be seen in Cases 21 and 55. In cases in which a series of transfusions have been given elsewhere previous to the examination of the blood, there is the possibility of the presence of surviving Group IV corpuscles. Usually the count of unagglutinable corpuscles before transfusion with an unagglutinable blood is below 70,000 corpuscles for each cubic millimeter of blood.

Case 4 of the eight cases presented by Wearn is not included in the tabulation. It was a transfusion of 300 c.c. of Group IV blood from a patient with pernicious anemia into another patient with pernicious anemia, who was in Group II. Since neither the initial count of the unagglutinable corpuscles nor the corpuscle content of the blood which was transfused was determined, no conclusion could be drawn from it. I included all of my published cases except those that are given as instances of the failure of transfused blood to remain in the circulation.

In all of my cases and, with the exception of Case 6, in all of Wearn's cases, there tends to be a 10:1 relationship between the number of hundred cubic centimeters of blood transfused and the number of millions of unagglutinated corpuscles for each cubic millimeter found in the circulation of the recipient as a result of the transfusion. This 10:1 relationship results when the recipient's blood volume is 5,000 c.c., and the count of the transfused blood is 5,000,000. A smaller corpuscle count in the transfused blood would decrease the number of unagglutinable corpuscles found in the circulation, and increase the first figure of the proportion; a smaller blood volume of the recipient would increase the second figure. In most instances both of these factors have operated simultaneously, as the blood used for transfusion was diluted by citration, and the patients had a low blood volume.

In Cases 7, 26, 43 and 50, in which the counts after transfusion are exceptionally low, a consideration of the data at hand would indicate that this can be accounted for by the higher blood volume of the patients and the lower count of the blood transfused. That in these apparent exceptions there is a relationship between the unagglutinable corpuscles found in the circulation after transfusion and the probable blood volume of the recipient, soon becomes evident when a blood volume estimate is made from the degree of dilution of the transfused blood as it occurs in the circulation, and a blood volume percentage is deduced from it. This has been done in Cases 7, 26, 43 and 50, using the following equation; the blood volume per cent. $= \frac{\frac{C}{U_2-U_1} \times T}{W}$, where T is the amount of blood transfused; C, the number of corpuscles in the transfused blood; $U_2 - U_1$, the difference between the count of unagglutinable

corpuscles before and after transfusion; and W, the weight of the patient. The results are as follows: In Case 43 ($W = 74$, $T = 0.42$, $C = 4.08$, $U_2 - U_1 = 0.248$) the calculated blood volume percentage was 8.4. In Case 7 ($W = 61.5$, $T = 0.5$, C was assumed to be 4.5 as the blood was citrated and the donor was a man, $U_2 - U_1 = 0.38$) the calculated blood volume is 9.7. In Case 26 ($W = 70.5$, $T = 0.46$, $C = 4.53$, $U_2 - U_1 = 3.46$) the calculated blood volume percentage was 8.5. In Case 50 ($W = 69$, $T = 0.5$; C was assumed to be 4.0 as the blood was citrated and the donor was a woman, $U_2 - U_1 = 0.35$, U_1 assumed to be 0.043) the calculated blood volume percentage is 8.3. These are within the blood volume percentages reported as normal by the vital dye method.[4] They would seem to be probable blood volumes for these cases, even for the two cases of pernicious anemia (Cases 26 and 43), for these patients were fairly well in spite of a low red count, and increases in plasma volume have been shown by Keith [5] to occur in cases of pernicious anemia which may more than compensate for the reduced corpuscle volume.

Applying the method of evaluating the counts of the unagglutinable corpuscles after transfusion by considering the possible dispersal of the blood transfused through the circulation of the recipient to the figures given by Isaacs,[1] it will be found that they are abnormally small, even the first day after transfusion. In the first case [6] 400 c.c. of blood having a count of 5,000,000 was transfused. The patient weighed 50.2 kg., and the greatest increase in the number of unagglutinable corpuscles that appeared after transfusion was 158,000 for each cubic millimeter. In order that the transfused blood should be so diluted by the recipient's blood that the count of the transfused corpuscles should be as low as this, it would be necessary for the recipient to have a blood volume which was 25.2 per cent. of her body weight. In the second case, 650 c.c. of blood with a count of 6,000,000 was given. The recipient's body weight was 51.4 kg. and the increase in the count of unagglutinable corpuscles, resulting from transfusion, was 105,000 for each cubic millimeter. These figures would indicate that the patient's blood volume was 75.5 per cent. of her body weight. As these blood volume percentages are impossible, it is very evident that the blood corpuscles transfused are not accounted for by unagglutinable corpuscles found by Isaacs one and two days after transfusion, while in my observations,

4. Keith, N. M.; Rowntree, L. G., and Geraghty, J. T.: A Method for the Determination of Plasma and Blood Volume, Arch. Int. Med. **16**:547-576 (Oct.) 1915.

5. Keith, N. M.: The Total Circulating Volume of Blood and Plasma in Cases of Chronic Anemia and Leukemia, Am. J. M. Sc. **165**:174-184 (Feb.) 1923.

6. Isaacs, R.: Personal Communication.

and I judge in those of Wearn, the transfused blood can be quantitatively accounted for during many days, from fifteen to thirty after the transfusion.

If Isaacs' technic is comparable to that used by Wearn and by me, his two cases must be abnormal, as an immediate elimination of transfused blood is indicated. If an error suggested by Isaacs in my technic, that of a retention of the sticky young cells in the tube in which the blood is allowed to agglutinate, is a factor, then the discrepancy between his two cases and the forty-eight presented by Wearn and by me, becomes greater instead of less.

Isaacs has changed the technic from a macroagglutination technic to a microagglutination technic. By the method, as I originated it,[7] a measured amount of serum and corpuscles was expelled into a Wassermann tube and shaken during the course of incubation to avoid inclusion of the unagglutinable corpuscles in the clumps formed. The time given was forty minutes with light shaking every ten minutes, but, as a matter of fact, agglutination is complete in a shorter time. After agglutination is complete, the free corpuscles are suspended by about twenty light shakes, and a sample is placed on the hemocytometer, and, immediately after settling, the count is made. By the modification of Isaacs, minute amounts of corpuscles and serum are measured; they are immediately placed on the counting chamber in which agglutination proceeds. By this method agglutination is not always complete in nine hours. Since the relative amounts of serum and corpuscles used by the two methods are comparable, this great lag in the procedure of agglutination must indicate that Isaacs has introduced a new factor. This might be the comparative lack of movement of the corpuscles, or it might be their tendency to adhere to the glass of the counting chamber to which they are greatly exposed. As the corpuscles have an opportunity to settle before they agglutinate, any of the young corpuscles described by Isaacs as sticky would necessarily stick where they settle, so that their failure to agglutinate would be evidence of their stickiness, but not necessarily of their unagglutinability. As by my technic I found low counts of 30,000 and 31,000 for each cubic millimeter of blood for the unagglutinable corpuscles before transfusion, in two cases of hemolytic jaundice, and as in the latter case the reticulocytes numbered 270,000 for each cubic millimeter of blood, I do not believe that the reticulocytes are intrinsically unagglutinable. Objection may be made to Isaacs' modification of the technic in that the insurance against inclusion of the unagglutinable corpuscles derived from transfusion in the clumps

7. Ice box incubation, which was included in the first description of the technic, was immediately retracted, as it was found to introduce irregularities attributed to false agglutination.

of the agglutinable corpuscles of the patient's blood has been removed, and in that it is not possible, by resuspension, to distinguish between the mechanical aggregation which tends to occur in some bloods, and true agglutination.

Since by my technic I have not found these young cells of Isaacs or the reticulocytes among the unagglutinable corpuscles after a transfusion of unagglutinable blood, and have only found them in the residue of native unagglutinable cells in the same proportion in which they were present in the whole blood, I do not believe that they have been a factor in my data.

The data on two unpublished cases which came under my observation, in which patients in Group I received Group II and Group IV transfusions, demonstrate that the type of the "unagglutinable cell" changes with the type of blood transfused. The blood of these patients, following Group II transfusions, in each case maintained its original agglutinability to Group IV serum, but there appeared and remained in the circulation for several weeks cells that were unagglutinable to Group II serum. One of these patients subsequently received a Group IV transfusion, which resulted in an immediate increase in the cells which were not agglutinable by Group IV serum. This increase was maintained for over two weeks. Since the type of the "unagglutinable cell" changed with the type of blood transfused, the possibility that these cells were derived from the host would seem to be ruled out.

So far as Isaacs' work with the blood of dogs is concerned, I do not consider that it is applicable to the question. The existence of isoagglutins in the blood of dogs is not established. By those workers who consider that they have found them they are admitted to be weak and inconstant as to type.[8] In my opinion they are unfit for separating mixtures of bloods.

SUMMARY AND CONCLUSION

Reasons are given, based on a large amount of data already published, which I consider prove that Isaacs is incorrect in his conclusion that "the use of agglutination in recognizing the cells of a donor in a mixture of two bloods in a transfused patient is of little value in from two to three days."

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8. Ottenberg, R.; Kaliski, D. J., and Friedman, S. S.: Experimental Agglutinative and Hemolytic Transfusions, J. M. Res. **28**:144, 1913.

DETERMINATION OF THE RESISTANCE OF LEUKOCYTES *

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INTRODUCTION

Certain constant results have been reported and much interest awakened within recent years in the study of the fragility of erythrocytes. It is not expected that similar findings will prevail in work on leukocytes, which are regarded as approaching more nearly to typical human tissue in structure and function than erythrocytes.

Academically, the observation and measurement of particular reactions of leukocytes as an example of living tissue are of definite interest, but from the clinical standpoint special attention is aroused by the finding of certain reactions by Mauriac, Carbonat and Moureau that are claimed to indicate the prognosis of many diseases.

Mauriac, Carbonat and Moureau [1] have done the primary work of importance in the field of leukocyte fragility. An outline of their work follows:

Mauriac,[2] in 1916, devised a technic for the determination of the resistance of leukocytes by exposing blood to a hypotonic aqueous solution of 0.206 per cent. sodium citrate and 0.3 per cent. sodium chlorid concentrations. He renewed the solution frequently because of the growth of molds. Three drops of blood were drawn into a Pasteur pipet and transferred into 0.5 c.c. of the before-mentioned solution. The mixture was then shaken and permitted to stand undisturbed for one hour. A clean slide was coated with a film of the suspended cells and dried for one-half an hour at room temperature. The preparation was stained, unfixed with saturated aqueous solution of methylene blue, washed gently in water and dried. The white cells were then counted.

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1. Mauriac, P.; Carbonat, P., and Moureau, M.: Compt. rend. Soc. de biol. **22**:816, 1919; Moureau, M.: Récherchés experimentales sur la fragilite leucocytaire, Théses de Bordeaux **62**:8, 1919; Mauriac, P., and Moureau, M.: Compt. rend. Soc. de biol. **83**:544, 1920.

2. Mauriac, P.: Ann. de méd. **3**:370 (July) 1916.

Mauriac was unable to differentiate the type of white cell by this method. The resistant ones appeared clean-cut in outline and the fragile cells hazy in form and pale blue. He established an arbitrary "Index of Fragility," namely, the number of resistant cells divided by the number of fragile cells. He found a decrease, or higher index, of fragility, with or without a leukocytosis in patients progressing to a favorable conclusion at certain critical periods in particular diseases, namely, immediately prior to (1) the clearing of the spinal fluid in cerebrospinal meningitis, (2) the clearing of the marked albuminuria and casts in the urinary sediment in nephritis and (3) the crisis in pneumonia. This diminution of fragility in cases presenting good prognosis he called an "Oscillation of Defense." Cases of typhoid fever, bronchitis, asthma and septicemia were likewise studied, but in these diseases the reactions were not so sharply defined as in the before-mentioned group. Other types of reactions than the "Oscillation of Defense" were observed. Mauriac found that an increased leukocyte fragility, as particularly noted in cases without an increasing leukocytosis, at any stage in a disease was an indication of poor general resistance and death frequently occurred. With each relapse of an infection, if the outcome was favorable, the fragility of the leukocytes fell, rising again gradually to normal as the patient recovered.

Mauriac, Carbonat and Moureau,[1] in 1919, utilized a slightly different technic in experimental work on the fragility of leukocytes. They noted very slight variation in normal rabbits. They constructed an index of fragility, namely, $n \times 100/N$, the "n" representing the number of fragile cells and the "N," the number of resistant cells. They employed the following technic: blood was drawn into a standard leukocyte counting pipet and diluted with an 0.1 per cent. aqueous solution of sodium citrate, 0.05 per cent. of sodium chlorid and 0.02 per cent. of methylene blue. The pipet was shaken and the cell suspension counted after a fixed period.

Three types of reactions were observed: (1) Succeeding the injection of turpentine, antidiphtheric serum, typhoid vaccine or colloidal metals in rabbits, Mauriac's "Oscillation of Defense" was demonstrated in those animals which showed favorable progress; (2) succeeding the intravenous injection of neoarsphenamin a leukocytosis was observed accompanied by an *increased* fragility and the authors suggested some association of this phenomenon with certain arsphenamin reactions in patients; and (3) succeeding the injection of various bacterial cultures and toxins, in those cases in which an increased fragility persisted in accompaniment with a normal leukocyte count or a leukopenia, the outcome was invariably fatal. But in the instance of the primary increased fragility

shifting permanently to a diminished fragility, all other conditions being similar, the outcome was not always fatal.

For certain reasons, to be outlined below, a modification of the methods utilized by Mauriac et al. has been made and used in the observations reported in this paper. Since the findings generally agreed with those of the before-mentioned investigators, it is not believed that these modifications detrimentally affected the results.

METHOD

A drop of blood is drawn into a leukocyte counting pipet to the mark "1," and the diluting fluid, consisting of 0.1 gm. sodium citrate and 0.05 gm. sodium chlorid to each hundred cubic centimeters of distilled water, is drawn up to the "11" mark. The pipet is gently rotated while the fluid is drawn up. It is not shaken, but slowly rotated while the mixture is blown into a test tube, of from 5 to 8 mm. internal diameter and from 3 to 6 cm. in length, which preferably has been lined with paraffin. The test tube containing the diluted blood is corked, preventing evaporation which would cause an alteration of the concentration, and is then gently agitated for one minute to insure an even distribution of the cells throughout the solution. It is then allowed to rest undisturbed for exactly two minutes more, during which period the same pipet is washed out with water and the barrel refilled to the point "1" with 9 per cent. sodium chlorid solution. At the expiration of the total three minutes the 9 per cent. sodium chlorid is blown into the solution, the entire mixture drawn once into the pipet and then expelled into the test tube. One drop of 1.0 per cent. aqueous solution Niagara blue or 1.0 per cent. trypan blue is then added to the contents of the tube, which is gently shaken once or twice and then recorked. A large drop of the solution is taken from the test tube, placed on a smooth glass slide and covered with a No. 0 or 1 thickness cover-slip which should not contain marked ridges or other irregularities and should measure from 1.5 by 1.5 cm. to 2.5 by 2.5 cm. The preparation is then rimmed with melted paraffin. The relative number of stained and unstained granular and nongranular white cells is counted in the proximal, central and distal portions of the preparation, running from one margin to the other of the cover-slip. The counting is completed within fifteen minutes from the time that the blood is diluted. Fragile cells have diffusely blue stained nuclei and the resistant cells are colorless and refractile. The counting of from two to three hundred white cells is facilitated by the ability to disregard erythrocytes through their early laking. The resistant and fragile white cells are distinctive, and one difficult to classify is very rarely encountered, actually less than 1 per cent.

COMMENT ON METHOD WITH FACTORS CAPABLE OF INCREASING THE EXPERIMENTAL ERROR

It is appreciated that with the ultilization of whole blood, many variable factors are introduced, some of which are later shown to have some appreciable effect in varying the number of fragile leukocytes by the method outlined in the foregoing. So far as present methods have progressed, it is apparently impossible to obtain pure leukocyte suspensions by the method of obtaining erythrocyte suspension without an indeterminate amount of trauma. The magnitude of this factor of trauma was demonstrated in an attempt to make such a leukocyte cream. Citrated blood was centrifugated in tubes with long, narrow necks so that a relatively high column of leukocyte cream was formed. This was pipetted off and suspended in Locke's solution. While this suspension was practically free of serum and erythrocytes, although not free of platelets, there were variable numbers of white cells injured prior to any exposure to hypotonic solution as determined by the diffuse staining of such cells by 1 per cent. aqueous Niagara blue solution. The tendency to an increase of the susceptibility of the apparently uninjured cells to hypotonicity was a definite possibility not determined here, but presenting another variable factor.

Pappenheimer [3] showed, in his work on rat lymphocyte suspensions, that certain colloids had a protective action on the cells when exposed to hypotonic Locke's solution. Table 4 summarizes the work that was done by me with 1 per cent. gelatin, Niagara blue, and acacia, in hypotonic sodium chlorid solution. In these experiments on human blood cells, gelatin and Niagara blue were active in the prevention of the destruction of cells in the hypotonic salt solutions. Pappenheimer included serum among the protective substances. By assuming a parallel situation in vivo it is possible that individual fluctuations in the proteins of the blood may be important factors, causing irregular determinations in investigations on the fragility of leukocytes which are not freed from erythrocytes and plasma. The control of the exact influence which these factors have on the fragility determinations must be left for future investigation.

The cell reactions to a hypotonic solution are complex phenomena depending on (1) the constituency and permeability of the cell membrane, (2) the difference in osmotic pressure of the solution and the protoplasm, and (3) certain other factors of vitality of the individual cell such as possibly a variation of the expansibility of the cell membrane causing it to burst at a certain point of distention. Much work has been done by Osterhout, Lillie, Overton, Loeb, Kuhne (quoted from

3. Pappenheimer, A. W.: J. Exper. Med. **25**:633 (May) 1917.

Bayliss [4]) and others, in the determination of the general properties of protoplasm and cell membranes. As in the instances of the erythrocyte fragility, it is assumed that there are undetermined vital factors of resistance which are variable. Such factors may eventually be explained on the basis of known physical and chemical properties in which the resistant and the fragile cells differ. Such may be compared to the way in which other processes, as, for example, the fertilization of ova, formerly explained by purely "vital" factors, have been partially established on a definite physical or chemical basis.

The succeeding technical factors discussed, namely, physiologic balancing of a solution, toxicity of the anticoagulant, and trauma, are apparently well controlled by their exact duplication with each leukocyte fragility determination, and may therefore be disregarded in comparison of various results in which the same technic is employed.

1. For simplicity a solution of sodium citrate in a solution of sodium chlorid has been used, even though this introduces injurious influences other than those involved in the use of a physiologically balanced hypotonic solution. Ringer,[5] Locke [6] and others have shown the importance of a balanced solution in the maintenance of living tissue, and Pappenheimer showed that fewer lymphocytes were destroyed by exposure to hypotonic Locke's solution than in dilute sodium chlorid.

2. Unger,[7] Drinker [8] and others have shown the existence of a slight toxicity for human cells of dilute sodium citrate solutions. It is one of the least toxic of anticoagulants, however, and is here used in the minimum concentration, namely, 0.2 per cent., to prevent clotting (Mauriac, Carbonat and Moureau [1]).

3. The amount of handling and the kind of instruments used must be constant. Evans [9] has shown that very slight mechanical trauma destroys living cells in vitro. Having minimized the trauma, the standard is carefully maintained.

The percentage of sodium chlorid in the diluting solution, namely 0.5 per cent., agrees with that of Carbonat, Mauriac and Moureau. This percentage was determined independently in the present work, as shown in Table 1. The objective in fixing this sodium chlorid concentration was the establishment of a normal ratio of injured to uninjured cells. This ratio should shift in either direction to a maximum degree with

4. Bayliss, W. M.: Principles of General Physiology, New York, Longmans, Green & Co., 1920, chapter V.
5. Ringer, S.: J. Physiol. **4**:222, 1882-1883.
6. Locke, F. S.: Zentralbl. f. Physiol. **15**:490, 1901.
7. Unger, L. J.: The Deleterious Effect of Sodium Citrate Employed in Blood Transfusion, J. A. M. A. **77**:2107 (Dec. 31) 1921.
8. Drinker, C. K., and Brittingham, H. H.: The Cause of the Reaction Following Transfusion of Citrated Blood, Arch. Int. Med. **23**:133 (Feb.) 1919.
9. Evans, H. M.: Personal communication.

abnormal specimens. The time of exposure of the cells to the solution should be long enough for convenience in handling and short enough to precede the distintegration of the dead cells. Three minutes' exposure and a solution of 0.05 per cent. sodium chlorid was considered the optimum ratio.

Hypotonicity offered an ideal injuring medium, from the standpoint that the duration of its activity may be easily controlled. At the end of the desired period, to the amount of one-tenth of the total volume of the solution, 9.0 per cent. sodium chlorid was added by measuring it in the barrel of the same white cell pipet. This brought the entire concentration of sodium chlorid to approximately 0.9 per cent. This is recognized as approximately isotonic for human tissue, and in such a solution it was found that a sealed blood cell suspension did not alter in per-

TABLE 1.—*Factors Determining the Destruction of Leukocytes with Hypotonic Solutions. Effect of Time at Various Concentrations of Sodium Chlorid in an 0.1 Per Cent. Sodium Citrate Solution*

Concentration of Sodium Chlorid, per Cent.	Percentage Destroyed in							
	3 Min.	5 Min.	10 Min.	15 Min.	20 Min.	30 Min.	40 Min.	60 Min.
0.0	88.4	93.0	96.5	100.0	....	....	....	....
0.02	90.0	94.0	97.0	100.0	....	....	....	....
0.04	75.6	90.2	92.4	93.4	97.0	100.0	....	....
0.05	50.0	67.8	84.4	81.7	89.8	92.6	100.0	....
0.06	44.0	65.6	69.9	72.8	76.0	81.2	81.2	82.5
0.08	28.4	39.6	40.5	48.1	57.5	62.4	66.0	....
0.10	28.0	32.4	34.6	38.7	....	....	....	67.2

The range of experimental error, as may be noted from fluctuation in counts on the same normal (Table 6), is greater than the apparently negative variations in Table 1, i. e., 0.05 per cent. in 10 minutes and 15 minutes.

centage of injured cells over 3 per cent. in two and a half hours. Leukocyte pipets furnish a ready source of accurate measurement of these small volumes, although at no time in this technic is the pipet used, as in blood counts, for shaking and distributing the cells because of the unquestioned trauma which the glass bead would inflict.

Nontoxic dyes, such as Niagara blue or trypan blue,[10] are desirable. These dyes readily stain the nucleus of injured or dead cells. The dense, diffuse staining of a cell nucleus is a fairly well accepted criterion of fatal injury of a cell. These benzidine dyes stain vitally only to a slight degree, if at all, and are so relatively nontoxic that they may be added in a concentrated aqueous solution. Thus, only a small volume of dye solution is necessary, in order to have an effective content of dye in the entire mixture for the rapid staining of cells without increasing the volume appreciably. The only disadvantage encountered in increasing the total volume of the mixture is dilution of the cell suspension which would prolong the period of counting.

10. Evans, H. M., and Shulemann, W.: Science, New York, **39**:433, 1914.

The chance irregular distribution on slightly ridged slides and coverslips of cells whose elasticity, power of adhesion and size unquestionably differ markedly, necessitates the counting in several regions of the preparation. The importance of not injuring the cells through evaporation and increase of the concentration of the solution necessitates the sealing of the preparation by petrolatum, or preferably melted paraffin. The solvents necessary to remove petrolatum or paraffin from glass dissolve the cement in most types of counting chambers, which precludes the routine use of these in place of plain slides.

All the glassware must be scrupulously cleansed in distilled water and dried prior to using, since small deposits of sodium chlorid and probably other soluble substances will materially affect the final results. The sodium chlorid and sodium citrate diluting solution must be kept on ice when not in use and renewed once a month.

Three factors liable to affect the destruction of the cells were investigated in the following brief experiments:

TABLE 2.—*Effect of Temperature Variation During the Exposure of the Blood to the Hypotonic Solution*

Temperature, C.	Percentage of Cells Destroyed with Hypotonic Solution, per Cent.	Percentage of Cells Destroyed with 0.9% Sodium Chlorid Solution (Control), per Cent.
12	32	0.5
24	50	1.0
35	85	0.0
45	96	0.0

1. *The Effect of Temperature.*—The glassware and solutions were brought to the temperatures noted in Table 2 and the technic carried out at these four temperatures. The increase of cell injury with higher temperatures was definitely observed. Therefore, to be comparable, all determinations of leukocyte fragility should take into consideration temperature variation.

In the following determinations on both normal and pathologic cases, the temperature of the room in which the tests were made varied within a few degrees of 25 C.

2. *Effect of Hydrogen-Ion Concentration of the Diluting Solution.*—The concentration of sodium ions and chlorin ions was kept approximately at 0.05 per cent. but the p_H was varied by the admixture of sodium hydroxid and hydrochloric acid. The results in Table 3 show a relatively slight variation, with the minimum fragility demonstrated in solutions of hydrogen-ion concentration from p_H 7.6 to p_H 8.4. After the addition of blood the mixture was necessarily buffered and consequently not the p_H noted in the table; for example, diluting solutions of

p_H 8.4 and p_H 6.4 when mixed with blood (p_H 7.45) [11] in the proportion before mentioned gave final determinations of p_H 8.17 + and p_H 6.57 + as determined by the electrometric method.

It has been observed that the p_H of the diluting solution would change on long standing in ordinary glassware from 6.8 to 8.3. It is therefore necessary to keep the diluting solution in a flask of hard glass, or one lined with paraffin.

TABLE 3.—*Effect of Varying the p_H of the Diluting Hypotonic Solution*

p_H *	5.6	5.8	6.2	6.6	6.8	7.0	7.2	7.4	7.6	8.2	8.4
Percentage of cells destroyed	56.0	52.0	52.0	54.5	48.5	54.5	50.0	48.5	42.0	38.0	38.0

* By indicator dye method.

Electrometric determinations of th p_H of the blood solution mixtures when, for example, the hypotonic diluting solutions were 8.4 and 6.4, was respectively 8.1 and 6.6 These two solutions of p_H 8.4 and p_H 6.4 were additional solutions not used in the foregoing series and therefore p_H 6.4 does not appear in this table.

3. The Protective Action of Certain Colloids (Table 4).—It was first found that if 1 per cent. Niagara blue was added to the blood at the same time as the diluting solution, only from 20 to 25 per cent. of the cells would be injured, instead of approximately 50 per cent., although the actual concentration of the sodium chlorid was diminished by the increase of the total volume.

This phenomenon suggested my repetition of Pappenheimer's work on the protective action of certain colloids, which has been previously discussed, and is summarized in Table 4.

TABLE 4.—*Effect of Certain Colloids in Protection of Leukocytes from Destruction by a Hypotonic Solution*

	Percentage of Cells Destroyed with Three Minute Exposure on Two Different Days (a) and (b)					
	Gelatin		Acacia Gum		Niagara Blue	
	a Per Cent.	b Per Cent.	a Per Cent.	b Per Cent.	a Per Cent.	b Per Cent.
Standard hypotonic solution containing 1 per cent. of the colloid	36	29	46	44	26	22
Standard hypotonic solution (control)	48	51	48	49	48	51

A similar protective action of a foreign substance was assumed in the following observation.

In using solutions from paraffined flasks, kept at room temperature for one week, there was a drop of from 54 to 36 per cent. of fragile cells in the resistance determinations on the same blood, at the beginning and at the end of the week, respectively. In this solution a heavy

11. Clark, W. M.: Hydrogen-Ion Concentration, Baltimore, Williams & Wilkins, 1920.

growth of bacteria, largely of the *B. fecalis-alkaligenes* type, was found Therefore, it was assumed that the actual bacteria or the products of their growth had a definite protective action.

The effect of the two before-mentioned factors, namely, hydrogen-ion concentration and protective action of growing bacteria, was proved by the entire absence of change in the destructive power of the solution when kept in paraffin lined flasks, which were constantly maintained in a refrigerator at about 10 C.

DETERMINATIONS ON NORMAL AND PATHOLOGIC BLOOD

Mauriac was unable to demonstrate by his technic the ratio of the various types of leukocytes destroyed. It is obvious that in certain instances the resistances of the different types of white corpuscles would be at variance. It is believed with the use of the technic herein outlined that, with moderately careful inspection, both in the case of the resistant forms and of the fragile forms, the coarsely granular myeloid cells may be differentiated from the finely granular lymphoid and large mononuclear cells. In two healthy men the ratios of coarsely granular cells to finely granular cells, among the resistant cells and the fragile cells of the same preparation, agreed very closely. Likewise, these were approximately the same ratios as the number of myeloid to other cells in Wright's stained smears of the same blood (Table 5). Similar determinations were made on one case of lobar pneumonia and on one of

TABLE 5.—*Normal Ratio of Coarsely Granular Myeloid Group to Finely Granular Large Mononuclear and Lymphoid Cells in the Fragile and in the Resistant Cells of Two Normal Adults*

Case		Resistant Cells, per Cent.	Fragile Cells, per Cent.	Total Leukocytes (Fixed Blood Film) per Cent.
1	Myeloid	64	62	65
	Other cells	36	38	35
2	Myeloid	58	62	66
	Other cells	42	38	34

chronic lymphatic leukemia, but in both these instances, as in the two controls, it was impossible to demonstrate a selection of a particular group of cells as being less resistant than the other.

The establishment of what is to be considered the figures for the percentage of fragile leukocytes has been only partially accomplished, as may be gathered from the rather wide variations obtained. Table 6 summarizes the findings on normal subjects. The subjects used were eighteen healthy adults, thirteen men and five women, between the ages of 21 and 35. Apparently from 45 to 55 per cent. of fragile cells may be considered the normal average, with 43 and 63 per cent. as the extremes. Daily and hourly fluctuations were observed in two subjects,

Cases 1 and 2, but the cause of these individual fluctuations is still to be learned, and perhaps may be associated with such total leukocyte fluctuations as occur following the taking of food.

TABLE 6.—*Determinations of Percentage of Fragile Cells in Normal Adults, Utilizing the 0.05 Per Cent. Sodium Chlorid—0.1 Per Cent. Sodium Citrate Solution with Three Minute Exposure* *

Case	Sex†	Percentage of Fragile Cells
1	♂	70 determinations; minimum, 44%; maximum, 62%; daily fluctuations within 10%; only 8 determinations were above 55% or below 40%
2	♀	54, 47, 52
3	♀	53
4	♂	58
5	♂	60
6	♂	45
7	♂	43
8	♀	55
9	♂	49
10	♂	58
11	♀	52
12	♂	49
13	♂	53
14	♂	51
15	♀	51
16	♂	53
17	♂	52, 54
18	♂	55

* Repeated determinations were made on different days except in several of the determinations in Case 1.
† In this column, ♀ indicates female, ♂ male.

Table 7 shows the pathologic cases studied. In the cases of pernicious anemia the fragility tended to vary inversely with the hemoglobin. The exception to this is Case 1, whose low resistance may be interpreted as a prelethal phenomenon.

With the leukocytoses due to infection especially, as shown in the pneumonia cases, there occurs an increased resistance of the recently extruded and relatively young leukocytes which the healthy blood-forming organs have sent out. This is Mauriac's "Oscillation of Defense," and whereas the leukocyte resistance has not been traced throughout the course of the disease, its subsidence with recovery in the Cases 12 and 13 indicates that it probably follows the same curve as Mauriac has traced and found to vary inversely as the absolute leukocyte count. It is especially important to note, as suggesting negative evidence in the use of leukocyte fragility as an aid in determining prognosis, that the two fatal cases showed an increased resistance when compared to the normal cases, and as such may be said to have had an "Oscillation of Defense." However, it is definitely demonstrable that none of the pneumonia patients recovering, showed a fragility of over 30 per cent. cells destroyed, while both of the fatal cases gave a greater percentage of cells destroyed. It must therefore be considered possible

to fix an arbitrary normal fragility for certain diseases with certain degrees of leukocytosis, which, if exceeded, will in all probability be indicative of a poor prognosis.

TABLE 7.—*Leukocyte Fragility in Adults with Certain Diseases*

A. Cases of Pernicious Anemia

Case	Percentage Fragile Cells	Hemoglobin, per Cent.	Erythrocyte Count, per C.Mm.	Leukocyte Count, per C.Mm.	Miscellaneous Features
1	89.7	15	.....	.....	One day prior to death (no evidence of blood regeneration at this time)
2	66.0	30	2,100,000	4,300	
3	60.0	40	.....	.....	
4	52.0	24	.....	.....	
5	50.0	18	848,000	3,700	Blood film shows 14% fragmented white cells

B. Cases with Secondary Anemia

6	27.0	40	.....	.....	Post hemorrhage
7 1st day	24.0	50	.....	.....	Sudden anemia after aspidium therapy for T. saginata; blood crisis with 14,000 per c.mm. nucleated red cells, 30% reticulated forms and many immature white cells
3d day	26.0	..	.....	.....	
5th day	38.0	..	.....	.....	
7th day	42.0	..	.....	.....	Gradual clearing of malaise with diminishing numbers of immature red cells in ten days though little change in hemoglobin
9th day	45.0	60	.....	.....	Continued improvement

C. Cases of Leukemia

Case	Percentage Fragile Cells	Hemoglobin, per Cent.	Total Leukocyte Count	Miscellaneous Features
8	75.0	45	150,000	Acute myelogenous leukemia (?)
9	22.0	..	86,000	Chronic myelogenous form in leukemic phase
(one month later)	27.0	..	4,800	Aleukemic phase
10	34.0	45	1,280,000	Chronic lymphoid leukemia

D. Cases with Leukocytosis

11	27.0	40	19,800	Nephritis, subacute
12 1st day	18.0	80	31,400	Lobar pneumonia
5d day	18.0	80	20,400	
6th day	48.0	..	16,800	During lysis
14 1st day	22.0	80	10,000	Lobar pneumonia (resolving)
2d day	28.0	..	.....	
4th day	40.0	..	8,200	After crisis
14	39.0	85	17,800	Lobar pneumonia complicated by miscarriage, death succeeding second day
15	32.5 (75% myeloid type, coarsely granular)	80	19,800	Lobar pneumonia; death on succeeding day
17	17.0	90	24,000	Lobar pneumonia; eventual recovery

The two instances of secondary anemia, Case 6 due to hemorrhage, and Case 7 probably due to a hemolytic toxin, showed the increased resistance expected with the content of recently extruded and probably relatively young cells in the blood stream. This is better shown in Case 7 than in Case 6, wherein a gradual return to normal ccurred concomitant with the fall in numbers in what are recognized as young forms of erythrocytes, namely, reticulated cells.

The two patients with a chronic type of leukemia demonstrate the same reaction as the infection leukocytoses, as would be expected from their exhibition of largely mature cells in the peripheral blood. The singe case of acute leukemia suggests the point, that in persistent, rapid expulsion of cells, the resultant forms are generally extremely immature, and as would be expected, are of poor resistance.

In these cases the bearing which the degree of leukocyte fragility has on prognosis is not especially striking, but certainly worthy of consideration. To establish the generalizations regarding the estimation of prognosis which are drawn by Mauriac, Carbonat and Moureau on a firm basis, it is necessary to prove the existence of the two major premises on which the generalizations depend. These are: (1) that the reaction of living cells to an artificial, unfavorable environment will resemble their reaction to the natural injurious agent of the disease under consideration; (2) that a certain tissue, the leukocytes in this instance, will parallel the resistance of other body tissues perhaps more vitally concerned in the maintenance of the proper functioning of the entire structure. This has not been done in this work. Therefore the conclusions are drawn reservedly on the basis of a certain constancy of results, which suggests the existence, at least in part, of the foregoing premises.

SUMMARY

1. A technic has been developed for the determination of the fragility of leukocytes by the microscopic examination of a measured amount of blood, which has been exposed to a hypotonic sodium chlorid, sodium citrate solution for three minutes and then stained with Niagara blue or trypan blue. The technic is believed to be of greater accuracy than the one described by Mauriac, Carbonat and Moureau and to be applicable clinically through its simplicity.

2. Certain influences liable to cause error have been investigated. It has been shown that the hypotonic sodium chlorid, sodium citrate solution must be kept continually on ice to prevent bacterial growth; that the determinations should be done at a constant temperature, preferably about 25 C., and that the solution should be kept in paraffin lined, or hard glass flasks to prevent alteration of hydrogen-ion content. It is appreciated from the protective influences that certain colloids, such as gelatin and Niagara blue, have on the leukocytes in hypotonic solution, that the variable constituency of the blood plasma may possibly play an indeterminate part in some of the results obtained; but it is impossible at present to obtain pure cell suspensions free of plasma without excessive trauma to the cells.

3. An attempt has been made to establish a normal percentage of fragile leukocytes. In fifteen adult males and three adult females under

probable constant conditions, there was a variation of from 43 to 63 per cent. of the fragile leukocytes, with 86.6 per cent. of the ninety determinations occurring between 45 and 55 per cent.

4. In six cases of lobar pneumonia with leukocytosis there was a marked reduction of fragility of the leukocytes in the four patients who recovered, and only a moderate reduction of the fragility in the other cases which terminated fatally.

The leukocyte fragility of five cases of pernicious anemia differed very slightly from the normal, except in a single patient shortly preceding death who showed increased fragility.

In acute and chronic myelogenous and chronic lymphoid leukemias an increased resistance was noted, except where many immature leukocytes were found in the peripheral blood when the resistance was decreased.

Two otherwise dissimilar cases, showing the common finding of severe secondary anemia in a stage of active regeneration, while sending out many young cells, demonstrated an increased resistance.

CONCLUSIONS

White blood cells recently delivered into the circulation have an increased resistance, excepting presumably, according to the present criteria of morphology, immature cells such as occur in acute leukemia. In patients who succumb to an infection or intoxication, there is evidence that the increased resistance is less marked than in those patients who are holding the infection or intoxication in check or overcoming it.

This partially confirms Mauriac's "Oscillation of Defense," which promises to be an additional factor in arriving at a prognosis in at least some of the acute, infectious diseases.

THE INTERPRETATION OF INCREASED BLOOD URIC ACID IN HYPERTENSION *

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REVIEW OF THE LITERATURE

It has been known for over a century that, after nephrectomy, urea accumulates in the blood,[1] and Bright (1834) was aware that this occurs in the disease first described by him. That uric acid may be increased in nephritis was discovered three-quarters of a century ago by Garrod [2] with the aid of his naive string test. Our modern knowledge of the blood chemistry in kidney disease was inaugurated when Strauss [3] demonstrated that the total nonprotein nitrogen of the blood is increased in asthenic uremia, and Widal [4] showed that the blood urea rises in this condition. Successful study of the constituents other than urea which make up the nonprotein nitrogen could only be carried out, after methods for their quantitative determination in small quantities of blood had been developed by Folin and his co-workers. It was then found that uric acid and creatinin also are often increased in renal insufficiency. Myers, Fine and Lough [5] believe that in progressive failure of renal elimination the first of the nitrogenous substances to be retained is uric acid, then urea, and only when the impairment of renal function becomes very marked does creatinin accumulate in the blood. In their opinion, an increase in the uric acid content of the blood in the presence of normal urea and creatinin values is an indication of beginning renal insufficiency, leukemia, gout, etc., of course, being excluded. Kraus [6] also regards the uric acid level of the blood as a fine indicator of retention, resulting from contracted kidneys. This view has found favor with French authors,[7] who speak of "l'uricémie d'alarme" which precedes urea retention.

The occurrence of an isolated uric acid retention as a precursor of the retention of other substances would be of both practical

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* From the Medical Service of the Montefiore Hospital.

1. Prevost and Dumas: Ann. de Chimie et de Physique **23**:1821.
2. Garrod: Med.-Chir. Trans. **31**:83, 1848.
3. Strauss: Die chronischen Nierenentzündungen in ihrer Einwirkung auf die Blutflüssigkeit. Hirschwald, Berlin, 1902.
4. Widal and Javal: Compt. rend. Soc. de Biol. **55**:1639, 1903.
5. Myers, V. C.; Fine, M. S., and Lough, W. G.: The Significance of the Uric Acid, Urea and Creatinin of the Blood in Nephritis, Arch. Int. Med. **17**:570 (April) 1916.
6. Kraus: Deutsch. Arch. f. klin. Med. **138**:340, 1922.
7. Jeanbrau, E.; Cristol, P., and Nikolitch, S.: J. d' Urol. **15**:249 (April) 1923.

and theoretical interest; of practical interest because it would furnish a very early warning of impending uremia, and of theoretical interest because it would unequivocally demonstrate that one of the "partial functions" of the kidney (that of uric acid excretion) can be damaged while the others, which are apparently vested in the same cells, remain intact. Quite obviously, a rise in the uric acid level of the blood does not necessarily mean that the ability of the kidneys to excrete this substance has been diminished. Thus the heightened uric acid content of the blood so often seen in leukemia is certainly to be attributed to the increased destruction of nucleoproteins and not to defective elimination. In the same hypertensive subjects who show an increased uric acid content of the blood, hyperglycemia is often to be found, which cannot be due to renal insufficiency, for the urine does not normally contain appreciable quantities of sugar, but must be explained as a derangement of carbohydrate metabolism correlated with the hypertension (hyperadrenalinemia [?], pancreatic arteriosclerosis [?]). Upham and Higley [8] found that in "early chronic interstitial nephritis" the ratio of the amount of uric acid in 100 c.c. of urine to that in an equal volume of blood is below normal. They state, however, that "in early chronic interstitial nephritis the height of the blood uric acid increase is not a guide to the degree of impairment of the renal concentration power." Hitzenberger and Richter-Quittner [9] studied the purin metabolism in two cases of essential hypertension with a normal blood urea but much increased uric acid in the blood. They found the endogenous factor to be slightly above normal, while any exogenous factor was promptly excreted, thereby demonstrating that the high uric acid content of the blood was not due to impaired excretion of this substance by the kidney.

CLINICAL MATERIAL STUDIED

We have studied the uric acid content of the blood in 110 cases of chronic hypertension. All of these cases were of the types ordinarily termed essential hypertension or chronic interstitial nephritis. Each had a diastolic blood pressure of 100 mm. of mercury or more. Blood uric acid values of 3.5 mg. per hundred cubic centimeters (determined by the method of Folin and Wu) were considered as presenting a hyperuricemia. Of the 110 cases it was found that forty-four, or 39 per cent., had 3.5 mg. or more of uric acid per hundred cubic centimeters of blood.

8. Upham, Roy; and Higley, H. A.: A Study of the Renal Concentration Power for the Uric Acid in Early Chronic Interstitial Nephritis, Arch. Int. Med. **24**:557 (Nov.) 1919.

9. Hitzenberger, K., and Richter-Quittner, M.: Wien. Arch. f. inn. Med. **2**:189 (Feb.) 1921.

Eighteen of these forty-three patients also had a blood urea nitrogen of over 20 mg. per hundred cubic centimeters. In the remaining twenty-five, constituting 27 per cent. of all not showing urea retention, the blood uric acid was 3.5 mg. or more per hundred cubic centimeters, while the urea nitrogen was under 20 mg. per hundred cubic centimeters. These patients with an increase in uric acid in the absence of urea retention are listed in the table.

These patients all suffered from the so-called essential hypertension, none being admitted to the hospital for any complaint due to renal insufficiency, but for the results of cerebral vascular accidents, myo-

Patients With Increased Uric Acid

No.	Diagnosis	Blood Pressure	Urea Nitrogen Mg./100 C.c.	Uric Acid Mg./100 C.c.	Sugar Mg./100 C.c.
1	Hypertension, general arteriosclerosis	190/120	18.2	4.5	110
2	Hypertension, cerebral hemorrhage	210/118	11.4	5.1	151
3	Hypertension, pulmonary emphysema	188/105	19.6	4.1	128
4	Hypertension.....	210/105	19.8	4.2	...
5	Hypertension, general arteriosclerosis	190/105	14.4	4.5	118
6	Hypertension, cerebral hemorrhage	260/160	13.4	5.1	103
7	Hypertension, angina pectoris	240/110	9.8	8.1	...
8	Hypertension, cerebral hemorrhage	205/130	9.5	4.5	92
9	Hypertension, encephalitis (residual)	210/135	15.1	6.7	113
10	Hypertension, pulmonary emphysema	170/120	17.2	5.0	...
11	Hypertension, cerebral hemorrhage, dyspituitarism.....	225/110	13.7	3.8	122
12	Hypertension, terminal cresol poisoning (suicide).....	200/110	12.8	5.5	...
13	Hypertension, diabetes mellitus	175/120	16.5	4.4	156
14	Hypertension, cerebral vascular accident..	240/130	12.7	3.7	124
15	Hypertension, diabetes mellitus	195/100	15.5	3.5	162
16	Hypertension, cerebral hemorrhage	250/140	15.1	4.0	129
17	Hypertension, diabetes mellitus, gangrene..	190/110	8.9	5.5	328
18	Hypertension, chronic cardiovascular disease.....	176/100	14.6	4.8	121
19	Hypertension, general arteriosclerosis	200/104	19.0	3.5	124
20	Hypertension, diabetes mellitus	240/100	16.4	5.7	350
21	Hypertension, adhesive pleurisy	190/110	18.2	4.6	91
22	Hypertension, mitral stenosis, auricular fibrillation.....	254/130	15.2	5.2	98
23	Hypertension, myocardial degeneration ...	152/120	10.9	3.8	154
24	Hypertension, cerebral hemorrhage, myocardial degeneration	200/110	9.8	4.2	91
25	Hypertension, mitral insufficiency.....	200/105	15.8	5.2	...

cardial insufficiency, diabetes, etc. Of the twenty-five patients only one developed uremia. This was Case 24. The patient, about three years after the above blood analysis was made, went into sudden coma which was diagnosed as "acute uremia." Unfortunately the blood chemistry was not studied at this time, so that even in this case it is not certain that the final coma three years later was of uremic nature. The other patients were in the hospital for from periods of a few weeks to over three years after the blood chemistry listed in the table was determined and none of them developed uremia. That the high blood uric acid does not portend nitrogen retention is shown in the following patients of the foregoing series, which were in the hospital for six months or more after the blood analysis listed in the table was made:

COURSE OF CASES

CASE 2.—This patient died two years after the foregoing determinations were made. Shortly before death the blood chemistry was: urea nitrogen, 15.0; uric acid, 3.5 mg. per hundred cubic centimeters.

CASE 6.—This patient, after remaining in approximately the same condition for a year and a half, died suddenly of acute cardiac failure with pulmonary edema.

CASE 7.—This patient, who had 8.1 mg. of uric acid per hundred cubic centimeters of blood, the highest figure of the cases listed above, was discharged a year later. Though his blood pressure was unchanged, the uric acid had fallen to 4.5 mg. and the blood urea was normal.

CASE 8.—This patient was discharged ten months later, with no evidences of nitrogen retention.

CASE 12.—This woman, one year after the foregoing chemistry was determined, had a blood urea nitrogen of 11.2 mg. per hundred cubic centimeters. Three years after, when in an unchanged condition, she committed suicide.

CASE 16.—This man died ten months later, his blood chemistry shortly before death being urea nitrogen, 21.0 mg.; uric acid, 2.3 mg. per hundred cubic centimeters.

CASE 23.—This patient was discharged six months later in an unchanged condition.

CASE 25.—This patient was discharged a year later in the same condition.

CASES 1, 2, 10 and 12.—These patients came to necropsy. In each case pronounced arteriosclerosis was present, being more marked in the smaller vessels of the kidneys. In each instance a large proportion of the secretory units (glomeruli and tubules) was intact, and apparently amply sufficient to carry on the excretory functions.

SUMMARY AND CONCLUSIONS

The observations here recorded indicate that these cases of essential hypertension with high blood uric acid, but normal amounts of urea in the blood, show no tendency to subsequent retention of urea and the parallel development of uremia. The patients followed for varying periods, from a few weeks to three years, did not develop renal insufficiency (except in one questionable instance) but suffered from the direct consequences of the circulatory strain, such as myocardial insufficiency or cerebral hemorrhage, or from some complication, most frequently diabetes mellitus. Anatomic investigation of the kidneys of four patients with high blood uric acid, who succumbed to these extrarenal manifestations, did not indicate that renal insufficiency was present. Despite the high content of the blood in uric acid these cases are "benign" so far as the kidney is concerned, and their chief dangers are cardiac failure or cerebral hemorrhage. Observation of the clinical course of these patients shows that the high blood uric acid does not presage impending renal insufficiency. The uric acid content of the blood fluctuates considerably, apart from diet, and apparently has no prognostic significance. We were unable to discern any differences in the symptomatology and course of the cases with hyperuricemia and

those with a normal blood uric acid. Like the hyperglycemia so common in hypertension, the increased uric acid content of the blood is due to metabolic causes and not to beginning renal insufficiency. When renal insufficiency sets in, the uric acid level of the blood rises, but it is then accompanied by a rise in the blood urea, which differentiates the hyperuricemia of renal origin from that accompanying hypertension in the presence of intact kidney function.

REPORT OF A FATAL CASE OF JUVENILE DIABETIC COMA WITH INSIGNIFICANT KETONURIA, AND WITH A LARGE AMOUNT OF ACETONE IN THE SPINAL FLUID *

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BROOKLYN

This fatal case of juvenile diabetic coma is reported because of the presence of several unusual features, namely, (1) the finding of only insignificant ketonuria in spite of extreme acidosis, (2) the presence of a large amount of acetone in the spinal fluid notwithstanding its absence from the blood, and (3) the failure of enormous doses of insulin to influence the blood sugar level.

REPORT OF CASE

The patient, a girl, aged 9 years, Jewish, was admitted in a state of coma to the United Israel Zion Hospital, Jan. 7, 1924. The family and past personal history were negative. The patient was in good health until three weeks prior to admission, when she began to suffer from increased frequency and volume of urination, pronounced thirst, itching of the skin and progressive loss of weight. Later she complained of slight disturbance of vision and headache, and two days before entering the hospital there was projectile vomiting not dependent on the taking of food.

Physical Examination.—The patient lay in profound coma, and the breathing was of the Kussmaul type. The complexion was sallow and cyanosed, and the body showed evidence of recent loss of weight. The breath had a strong acetone odor, the tongue was heavily coated, the gums were inflamed and the teeth showed considerable decay. There were excoriations about the vulva, evidently from scratching. The temperature was 101 F., pulse 130, respiration 42.

The urine had a specific gravity of 1.030, was acid in reaction, and contained 3 per cent. of sugar. There was a faint trace of acetone, but no diacetic acid. A second specimen gave the same findings with reference to these ketone bodies.

At the time of admission the blood chemical findings were as follows: Sugar 333, urea nitrogen 30, and creatinin 3.33 mg. per hundred cubic centimeters of blood. The carbon dioxid combining power of the plasma as determined by the method of Van Slyke and Cullen was 14.3 per cent. by volume.

The patient was treated with lavage of the stomach, forced fluids, and bicarbonate of soda solution by rectal drip and saline infusions. Immediately on admission, 40 units of insulin (H insulin-Lilly) were administered intravenously, followed by a similar dose three honrs later, and 30 units after another three hours. Altogether 110 units of insulin were given within twelve hours. In spite of this enormous dosage, there were no twitchings or other evidences of hypoglycemic shock. Neither was there any favorable response to the medication, and death occurred nine hours after the last dose of insulin. At the time of death, blood chemical examination gave the following figures:

* From the Pathobolic Service, United Israel Zion Hospital, Brooklyn, N. Y.

Sugar 333, urea nitrogen 75, creatinin 3 and uric acid 1.8 mg. per hundred cubic centimeters of blood. The reaction for acetone was negative. It is noteworthy that 110 units of insulin administered within a period of twelve hours had failed to affect the blood sugar level in the least.

The chemical findings in the spinal fluid drawn immediately after death were as follows: Sugar 300, urea nitrogen 60, creatinin 2.7 and uric acid 2.4 mg. per hundred cubic centimeters. A surprising finding was the presence of a very large amount of acetone, as judged by the reaction to Legal's test.

COMMENT

Severe diabetic acidosis without ketonuria is quite unusual, but authentic cases have been reported. Revillet,[1] in 1914, described a fatal case, clinically one of diabetic coma, in which no acetone was found in the urine and in which Gerhardt's test was negative. Rosenbloom,[2] in 1915, reported three cases of diabetic coma in which the urine showed no trace of acetone, diacetic acid or beta-oxybutyric acid, and contained a normal amount of ammonia nitrogen. McCaskey,[3] in 1916, described a fatal case of diabetic coma in which there was acetone in the urine, but no diacetic or beta-oxybutyric acid at any time. Other cases have been reported.

Starr and Fitz,[4] in 1924, reported a study on 114 samples of urine from eighteen diabetic patients as to their content of acetone bodies and undetermined organic acids, and they found that in about 10 per cent. of instances organic acids other than the acetone bodies were present in considerably higher than normal concentration. These authors believe that in certain cases of diabetic acidosis other organic acids than the ketones are encountered, and may play a part in the production of symptoms.

In the present case, the finding of a large amount of acetone in the spinal fluid, notwithstanding its absence from a simultaneous blood specimen, is fraught with possibilities and suggests the desirability of further work along this line.

The failure of large doses of insulin to influence favorably diabetic acidosis is not so unusual as to deserve special comment, but the fact that 110 units of insulin (H insulin-Lilly, which is 40 per cent. stronger than the older preparation) administered within twelve hours failed in any way to affect the blood sugar level would appear to be a rare enough finding to be worthy of record.

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1. Revillet: Coma chez une diabétique sans acétonurie, Lyon méd. **122**:815, 1914.
2. Rosenbloom, J.: A Form of Diabetic Coma Not Due to the Acetone Bodies, New York M. J. **102**:294, 1915.
3. McCaskey, G. W.: A Case of Fatal Diabetic Coma Without Diacetic or Beta-Oxybutyric Acid, J. A. M. A. **66**:350 (Jan. 29) 1916.
4. Starr, Paul; and Fitz, Reginald: The Excretion of Organic Acids in the Urine of Patients with Diabetes Mellitus, Arch. Int. Med. **33**:97 (Jan.) 1924.

SUMMARY

In the case of a comatose girl, aged 9 years, with the classical symptoms of diabetes mellitus dating back three weeks and with a markedly reduced carbon dioxid combining power of the plasma, the urine revealed only a faint trace of acetone and no diacetic acid. Notwithstanding the fact that there was no acetone in the blood, a very large amount of this substance was found in the spinal fluid. Although 110 units of insulin (H insulin-Lilly) were administered within a period of twelve hours, there was no reaction at all and the blood sugar level was not in the least affected.

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FATE OF HEXAMETHYLENAMIN IN THE BODY AND ITS BEARING ON SYSTEMIC ANTISEPSIS *

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SAN FRANCISCO

INTRODUCTION

Besides its common use as a urinary antiseptic, hexamethylenamin is alleged to be beneficial, when administered orally and intravenously, in various infectious diseases. Clinically,[1] it has been administered in this way in the treatment of typhoid (Ymaz; Loeper; Ymaz and Ayerza), pneumonia (Loeper; Clarke; Mikhailoff; Loeper and Grosdidier), nephritis (Loeper and Grosdidier; Loeper; de Matta), epidemic encephalitis (Thomas and Rendu), influenza (Loeper and Grosdidier; Michel), serofibrinous pleurisy (Loeper and Grosdidier; Michel), septicemia (Achard), typhus (Glutard), tuberculosis (Loeper and Wagner), prostatitis and epididymitis (Cooke), bronchitis (Mikhailoff; Never), and malaria (Plaschkes and Benkovic). A sound basis for the use of hexamethylenamin in various systemic infections appears to be lacking. However, it is conceivable that certain conditions might exist which would permit hexamethylenamin to exert an antiseptic action. These would be concerned with the liberation of formaldehyd in the blood and tissues.

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* From the Department of Pharmacology, School of Medicine, Stanford University.

* Thesis (Part II) submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

1. Ymaz: Semana méd. **29**:807-809, 1922. Loeper: Paris Letter, J. A. M. A. **70**:483, 1918. Ymas and Ayerza: Prensa Med. Argentina, **5**:215, 1918. Clarke, T. W.: Am. J. Obst. **72**:753, 1915. Loeper and Grosdidier: Bull. et mèm. soc. méd. d. hôp. de Par. **42**:566-569, 1067, 1073, 1918. De Matta: Amazanos Medical **1**:81, 1918. Thomas, A., and Rendu, H.: Paris méd. **11**:273 (Oct. 1) 1921. Michel: Presse méd. **27**:126-127 (March 10) 1919. Loeper and Grosdidier: Progrès méd. **33**:427, 1918. Achard: Progrès méd. **33**:385, 1918. Glutard: Bull. et mém. soc. méd. d. hôp. d. Par. **43**:545, 1919. Loeper and Wagner: Bull. et mém. soc. méd. d. hôp. d. Par. **42**:569, 1918. Cooke, E. S.: New York M. J. **116**:542 (Nov. 1) 1922. Neves, A.: Arch. de méd. d. enf. **21**:29 (Jan.) 1918. Plaschkes and Benkovic: Wien. klin. Wchnschr. **29**:1495, 1916. Other references along similar lines but not mentioned in the text are: Duthoit: Antisepsis in Gallbladder, Compt. rend. soc. biol. **89**:656, 1923. Von Takats, G.: Arch. f. klin. Chir. **125**:544 (Sept.) 1923; and discussions, Davis, E.: Intravenous Injection and Urinary Antisepsis, J. Urol. **11**:29 (Jan.) 1924.

Experimental evidence indicates that hexamethylenamin per se has no antiseptic qualities,[2] and that antisepsis is dependent upon the free formaldehyd liberated by hydrolysis, or decomposition, of hexamethylenamin, which in turn depends on the hydrogen-ion concentration of the medium. Although the degree of decomposition increases with an increase in the hydrogen-ion concentration, it has been reported by Trendelenburg [3] that an appreciable liberation of free formaldehyd occurs on the alkaline side of neutrality. When the drug is taken by mouth it appears that the excretion is incomplete. The incompleteness of excretion might be explained by decomposition of the drug in the body, resulting in the liberation of formaldehyd.

In view of these possibilities and of the favorable clinical reports, the subject was deemed worthy of extended study with the idea of determining, if possible, the rational basis of hexamethylenamin as a systemic antiseptic.

The present investigation, therefore, was planned along several lines: (1) A brief study of the urinary excretion of hexamethylenamin in human subjects was made in order to ascertain the extent of loss of hexamethylenamin in its passage through the body, and also the influence of gastric acidity on this; (2) the extent of hydrolysis of the drug with formaldehyd liberation in solutions and serum mixtures of known chemical reaction, including alkalinity, was ascertained; (3) the extent of decomposition in the blood of animals under conditions most favorable to this, namely, acidosis was tested; and (4) the excretion of formate in the urine of human subjects as an index of the decomposition of the drug over longer periods in the tissues was determined. These features are presented in this order, beginning with the excretory results, since excretion is unavoidably bound up with the fate of the drug in the body.

1. EXCRETION OF HEXAMETHYLENAMIN IN HUMAN SUBJECTS

In some preliminary experiments previously reported by Collins and Hanzlik [4] the quantitative excretion of hexamethylenamin in urine appeared to be quite variable. Falk,[5] who also reported variable excretion, claimed a lower percentage of excretion in nephritic than in normal persons, and made the suggestion that hexamethylenamin might be used as a test of renal functional efficiency. If the drug is partially hydrolyzed in the stomach and tissues, such a test would be of little use.

2. Hanzlik, P. J., and Collins, R. J.: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency Under Different Chemical and Biologic Conditions, Arch. Int. Med. **12**:578 (Nov.) 1913.

3. Trendelenburg: Biochem. Ztschr. **95**:146, 1919.

4. Collins, R. J., and Hanzlik, P. J.: J. Biol. Chem. **25**:231 (June) 1916.

5. Falk, K. G., and Sugiura, K.: J. Pharmacol. & Exper. Therap. **8**:39 (Jan.) 1916.

The experiments in this study were made on convalescent patients and healthy subjects. They received doses ranging from 1 to 5.2 gm. of hexamethylenamin by mouth. The urines were collected until free from the drug, and then they were subjected to quantitative estimation, by the distillation colorimetric method of Collins and Hanzlik.[4] In some experiments hourly administrations of water and hourly collections of urine were made to determine the influence of diuresis on the excretion. In other experiments, sodium bicarbonate was administered in order to test out the influence of gastric acidity. The results obtained are presented in Table 1.

TABLE 1.—*Quantitative Excretion of Hexamethylenamin in the Urine of Human Subjects*

Subject		Quantity of Hexamethylenamin and $NaHCO_3$ Administered	Per Cent. Hexamethylenamin Excreted	Total Volume of Urine Excreted, C.c.	Duration of Excretion, Hours	Diagnosis
H. W.	1 A	Hexamethylenamin 5.2 gm. constant H_2O intake	82.3	1,757	39	Convalescent (acute parotitis)
	1 B	Hexamethylenamin 5.2 gm. natural H_2O intake	40.0	3,485	41	
E. L.	2 A	Hexamethylenamin 1.0 gm. constant H_2O intake	32	2,470	20	Convalescent (varicella)
	2 B	Hexamethylenamin 1.0 gm. natural H_2O intake	65	1,044	20	
W. H.	3 A	Hexamethylenamin 1.0 gm. constant H_2O intake	66	1,638	21	Convalescent (measles)
P. H.	4 A	Hexamethylenamin 1.0 gm.	85	2,185	33½	Normal person
	4 B	Hexamethylenamin 1.0 gm. and $NaHCO_3$ 1 gm.	100	1,925	24	Normal person
M. D.	5 A	Hexamethylenamin 1 gm.	71	1,905	31½	Normal person
	5 B	Hexamethylenamin 1 gm. and $NaHCO_3$ 5 gm.	87	2,053	24	Normal person
P. H.	4 C	Hexamethylenamin 1 gm. and $NaHCO_3$ 5 gm.	86	2,100	24	Normal person
M. D.	5 C	Hexamethylenamin 1 gm. and $NaHCO_3$ 5 gm.	86	2,450	..	Normal person

Table 1 shows that the total excretion in subjects receiving hexamethylenamin alone was variable, ranging from 32 to 85 per cent. This confirms the results of previous investigators. The excretion lasted from twenty to thirty-three and a half hours with 1 gm. doses, and about forty hours with 5.2 gm. doses of the drug. The data also show that the total excretion was uninfluenced by diuresis. However, the administration of bicarbonate augmented the excretion. In the four experiments performed on two subjects, excretion before administration of from 1 to 5 gm. of bicarbonate was 71 and 85 per cent., after bicarbonate the excretion ranged from 86 to 100 per cent. In other words, neutralization of the hydrochloric acid of the gastric juice tended to increase the excretion of hexamethylenamin in virtue of the decreased hydrolysis of the drug before absorption. The experiments with bicarbonate were not extended further because of difficulties in determining definitely to what extent neutralization of the hydrochloric acid in the

stomach is accomplished. In any case, the median total excretion after bicarbonate in the majority (three) of four experiments still left 14 per cent. of the drug unaccounted for, indicating the influence of some other factors. The results obtained indicate that hexamethylenamin administered orally would be unreliable as an agent for testing renal functional efficiency.

Therefore, it is concluded that the urinary excretion of hexamethylenamin is rather variable, leaving from 15 to 68 per cent. of the administered drug unaccounted for. The loss is due in part to the destructive influence of gastric acidity, but is independent of the dosage, water intake and diuresis.

Higher doses might be expected to increase the quantity of drug excreted unless decomposition in its passage through the body occurs. This might occur despite the destructive action of the gastric acidity, which, however, is variable. The alkalinity of the intestine would have no part in the destruction. The data in Table 1 show that the total excretion of hexamethylenamin in urine was no greater with doses of 5 and 5.2 gm. than with doses of 1 gm. In fact, the higher dosage of 5.2 gm. resulted in smaller excretion, namely, from 40 to 86 per cent. as compared with a range of from 32 to 100 per cent. with 1 gm. doses. This suggests the possibility that some other factors besides those of the alimentary tract enter into the decomposition of the drug, namely, the tissues.

2. POSSIBLE RÔLE OF THE TISSUES

The rôle of the tissues in the decomposition, or hydrolysis, however small, might nevertheless help to explain the variable excretion, which is characteristic of the drug, and also the beneficial effects of hexamethylenamin administered intravenously, and by mouth, in various infectious diseases. That is, in the hydrolysis or decomposition of hexamethylenamin, liberation of formaldehyd occurs, and in the blood and tissues this product might momentarily exist before oxidation to formic acid. If free formaldehyd could be demonstrated, it would help to reconcile the alleged beneficial effects on a rational basis. On the other hand, if no hydrolysis of hexamethylenamin could be demonstrated under conditions of the blood and tissues, either by the absence of formaldehyd or of ammonia, or products of either of these, then the variability in the total excretion would have to be relegated to variable conditions in the alimentary tract. The same negative results would disprove the alleged beneficial effects of hexamethylenamin intravenously, or by any other channel of administration. Otherwise it would be necessary to postulate some mechanism of antiseptic action other than that generally accepted and there is no justification for this. Therefore, it was decided to determine to what extent, if at all, hexamethylenamin is decomposed under conditions of the blood and tissues.

3. HYDROLYSIS OF HEXAMETHYLENAMIN IN BUFFER MIXTURES

In studying the hydrolysis of hexamethylenamin it was thought desirable to secure indirect as well as direct experimental evidence. When the products of hydrolysis, i. e., ammonia and formaldehyd, were determined, the evidence was direct. When estimations of products derived from ammonia and formaldehyd, namely, urea and formic acid, were made, the evidence was indirect. Determinations of ammonia and formaldehyd were feasible in the experiments in vitro, but, for reasons that will be indicated later on, the experiments on animals necessitated estimations of urea and formic acid.

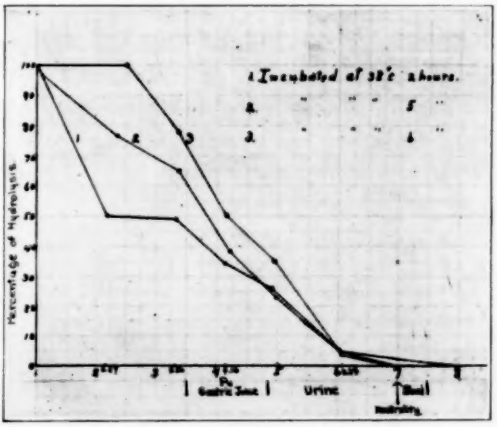

Chart 1.—Liberation of formaldehyd from, or hydrolysis of, hexamethylenamin in buffer mixtures, covering ranges of p_H in gastric juice, urine and blood.

Hydrolysis of hexamethylenamin in aqueous solutions of different p_H values was studied in the following manner: A series of buffer phosphate solutions of known p_H value for the range of from 5.3 to 8 and of citrate hydrochloric acid for the range of from 1 to 4.9, was placed in volumetric flasks of 100 c.c. capacity, and to each flask was added 0.2 gm. of hexamethylenamin. The mixtures were then incubated at 38 C., and at the end of definite time intervals samples were removed, and estimations of free formaldehyd were made directly by the phloroglucin colorimetric method of Collins and Hanzlik.[4] The results obtained are presented in the form of curves in Chart 1.

These show that the colorimetric method for estimating free formaldehyd was satisfactory for the purpose of this study and that the results (with buffers ranging from a p_H of 1 to a p_H of 7) of

Trendelenburg were verified. That is, the results indicate a hydrolysis of, with liberation of formaldehyd from, hexamethylenamin more or less proportional to the degree of acidity, being greatest in degrees of acidity higher than and corresponding to that of gastric juice, less in that of urine, and least at neutrality. The curves also show a slight amount of hydrolysis on the alkaline side of neutrality, that is, in the range corresponding to and higher than that of blood when incubation was continued for six hours.

Since the hydrolysis on the alkaline side is of the greatest interest, the experiment was repeated to obtain more detailed data in the p_H range of from 6.5 to 8.5, using phosphate mixtures of from 6.5 to 8 and borate hydrochloric acid for the 8.5 mixture. The results of this experiment are presented in Chart 2.

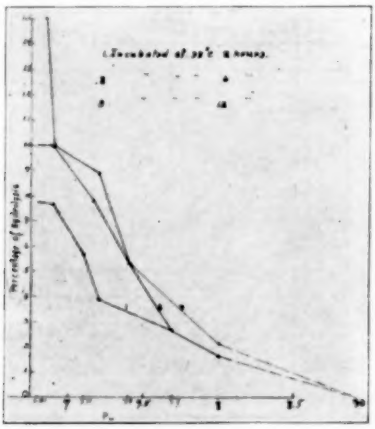

Chart 2.—Liberation of formaldehyd from, or hydrolysis of, hexamethylenamin in alkaline buffer mixtures (p_H range from 6.81 to 9.0).

It is seen that the hydrolysis of hexamethylenamin between $p_H = 8$ and $p_H = 7$ ranged from 0.15 to 0.67 per cent. after incubating for two hours, from 0.15 to 0.93 per cent. after incubation for six hours, and from 0.2 to 0.97 per cent. at the end of incubation for twelve hours. In this connection the mitigating influence of liberated ammonia was considered because the p_H values shifted to the alkaline side but was found to be negligible. That is, during the hydrolysis of hexamethylenamin there is a liberation of ammonia which might cause a shift of the p_H value of a given buffer mixture toward the alkaline side and so cause a diminished hydrolysis. This factor might be of importance in the p_H range of from 6.5 to 8.5. To determine the effect of liberated ammonia the p_H values of the mixtures were deter-

mined by the colorimetric method at the time of analysis for formaldehyd. It was found that the percentage decomposition of hexamethylenamin remained practically the same, and, therefore, the decrease in hydrolysis caused by a shift of the p_H value to the alkaline side due to the liberated ammonia was negligible.

The results obtained thus far indicate the possibility of formaldehyd liberation from hexamethylenamin in the blood and other body fluids, but the actual demonstration of the formaldehyd in the presence of protein might be different, and, therefore, requires simulation of the conditions in vivo more closely.

4. HYDROLYSIS OF HEXAMETHYLENAMIN IN BUFFER MIXTURES CONTAINING SERUM PROTEIN

Having shown that hydrolysis of hexamethylenamin is a function of the p_H and that there is an appreciable liberation of formaldehyd at p_H values greater ($p_H = 8.0$ and 7.4) than those of body fluids (blood,

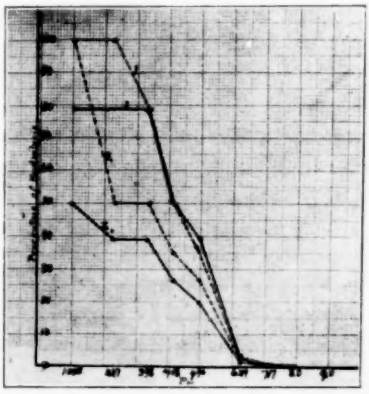

Chart 3.—Liberation of formaldehyd from, or hydrolysis of, hexamethylenamin in serum protein. Curves marked 1 indicate incubation for six hours at 38 C., and those marked 2, for two hours at 38 C. Solid lines represent hydrolysis in presence, and broken lines in absence, of horse serum.

cerebrospinal fluid, etc.) the effect of protein on the hydrolysis in the various buffer solutions was studied. The procedure was the same as in the case of buffer mixtures alone except that the buffers contained 5 per cent. horse serum. Typical results are presented in the form of curves in Chart 3.

The results obtained show that protein in the form of horse serum had a definite retarding influence on the hydrolysis, or decomposition, of hexamethylenamin at any given p_H level, and that liberation of formaldehyd was not demonstrable in truly alkaline buffer serum

mixtures. In view of the fact that the phloroglucin reagent is sensitive to formaldehyd in as low a concentration as from one to five million, any undetected formaldehyd in the buffer serum mixtures must have been negligible. On the other hand, considerable liberation occurred in the acid mixtures as would be expected, although quantitatively the liberation was less in the presence of serum protein than in the absence of it.

5. DECOMPOSITION OF HEXAMETHYLENAMIN IN THE CIRCULATION

Although the results with the buffer serum mixtures reduced the possibility of decomposition of, or formaldehyd liberation from, hexamethylenamin in the blood, it still could not be concluded that the drug might not be appreciably decomposed in the circulation and tissues because the conditions in the living organism differ from the experiments in vitro even in the presence of protein. Proteins, no doubt, are essential factors in mitigating formaldehyd detection. However, this is balanced by several factors, namely, by the hydrolyzing influence of ferments; by the fact that the tissues proper are, in reality, a trifle more acid ($p_H = 6.8$ or thereabouts) than the blood, and by the continuous exposure of hexamethylenamin to the decomposing influences of the tissues. These, in virtue of the circulation, are some of the considerations among others less understood, which enter into the possibility that hexamethylenamin may hydrolyze and liberate formaldehyd in the blood tissues. In disease conditions, in which the chemical reaction of certain tissues is such as to be favorable to the decomposition, as in the case of pneumonia in which lung extracts were shown to be acid ($p_H = 6.0$) by Lord,[6] formaldehyd might be freed, at least momentarily and locally. If hydrolysis could be demonstrated in the circulation of normal animals, there would not be much doubt of such hydrolysis (with formaldehyd liberation) in the diseased organs or tissues whose reaction is truly acid, or rendered acid, as by the induction of asphyxia. Hence, it was decided to proceed at once to test the proposition; whether or not hexamethylenamin is hydrolyzed in the circulating blood. The experiments on animals may now be described.

METHODS

The phloroglucin method, which is satisfactory for the estimation of small quantities of formaldehyd in colorless solutions, cannot be used for either the qualitative detection or quantitative estimation of formaldehyd in a colored medium such as blood. Low concentrations

6. Lord, F. T.: The Relation of the Pneumococcus to the Production of Acid in Fluid Culture Mediums and the Reaction of the Pneumonic Lung, J. A. M. A. **72**:1364 (May 10) 1919; **73**:636, 786, 1364, 1420, 1919.

of formaldehyd in blood cannot be removed quantitatively by dialysis nor by aeration, as shown by the following experiments: Formaldehyd in a concentration of from one to five thousand was vigorously aerated with ammonia-free air for three hours, and analysis of the original solution showed that 96 per cent. of the formaldehyd had not been removed. Aqueous solutions of formaldehyd in concentrations of from one to five hundred thousand were dialyzed for periods ranging from one to twenty hours and the dialyzate never gave more than a faintly positive test for formaldehyd, using the phloroglucin reagent. These results are in exact agreement with numerous, unpublished experiments of Hanzlik in which both aeration and dialysis failed to give quantitative removal of formaldehyd from aqueous solutions and from serum.

In view of these difficulties, evidence for the presence of formaldehyd in the body fluids after administration of hexamethylenamin had to be obtained indirectly. The end products of hexamethylenamin hydrolysis in the organism are sodium formate, and ammonium salts, which are converted to urea. Consequently, increases in the blood urea nitrogen, and the presence of formic acid were used as indices of hexamethylenamin hydrolysis in the circulation. The urea nitrogen estimations were made by the Van Slyke and Cullen [7] modification of Marshall's method. The ammonia was driven over by aeration and estimated colorimetrically in the usual way. The formic acid was estimated by a method previously described.[8] Briefly, this consists of the removal of interfering substances by picric acid and estimations of the formic acid in the filtrate by causing reduction of mercuric chlorid to calomel, which is then determined volumetrically by means of standard solutions of iodin and of thiosulphate. The experiments fell into two main groups: (1) urea-nitrogen, and (2) formic acid estimations in the blood.

CHANGES IN UREA NITROGEN OF THE BLOOD

For a study of these changes experiments were made on rabbits and dogs. The results on rabbits will be described first.

A sample of control blood was removed by cardiac puncture and the urea nitrogen estimated. Then 5 c.c. of 10 per cent. hexamethylenamin was injected into an ear vein, and subsequently samples of heart blood were removed at intervals of fifteen minutes. Urea estimations were made immediately after withdrawal of the blood. The results obtained on the five rabbits that were used are summarized in Table 2.

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7. Van Sylke, D. D., and Cullen, G. E.: A Permanent Preparation of Urease, and Its Use for Rapid and Accurate Determination of Urea, J. A. M. A. **62**:1558 (May 16) 1914.

8. De Eds, Floyd: J. Lab. & Clin. Med., 1924 (in press).

It is seen that the results are somewhat variable. They indicate insignificant increases and decreases in the urea nitrogen content of the blood as a result of the injection of hexamethylenamin. Only two rabbits (Nos. 3 and 4) showed increases in urea nitrogen, while the other rabbits (Nos. 1, 2 and 5) showed corresponding decreases. The decreases in urea nitrogen in Rabbits 1, 2 and 5 might be accounted for by variations accompanying the tendency to the establishment of an equilibrium between the blood and tissues. In the rabbit experiments no allowance was made for possible changes in blood volume. The injection of hypertonic solutions of hexamethylenamin may cause a washing out of urea from the tissues, and so account for the increased urea values shown by Rabbits 3 and 4.

TABLE 2.—*Changes in Urea Nitrogen Content of the Blood in Rabbits Produced by Intravenous Injection of Hexamethylenamin*

Rabbit	Dose of Hexamethylenamin Injected, Gm.	Mg. Urea Nitrogen in 100 C.c. of Blood	
		Before Hexamethylenamin	After Hexamethylenamin
1	0.5	10.9	8.7 end of 55 minutes
2	0.5	10.0	9.55 end of 45 minutes
3	0.5	9.5	10.58 end of 15 minutes
			10.26 end of 30 minutes
			10.36 end of 45 minutes
4	0.5	9.73	11.24 end of 15 minutes
			10.75 end of 30 minutes
			10.26 end of 45 minutes
5	0.5	11.7	9.21 end of 15 minutes
			9.30 end of 35 minutes
			9.35 end of 60 minutes
			8.96 end of 105 minutes

More marked increases in urea content of blood might be brought about by inducing asphyxia which lowers the p_H of the blood, or in other words, causes acidity, and, therefore, should result in increased hydrolysis of hexamethylenamin. Since experiments involving asphyxia over prolonged periods are more suitably and successfully carried out on dogs, the remainder of the experiments were done on these animals.

The dogs received 25 c.c. of 40 per cent. hexamethylenamin intravenously. Blood from the right femoral artery was removed at intervals, and collected under clean liquid petrolatum containing a little dry sodium oxalate for prevention of clotting. Estimations were made of urea nitrogen (by the same method before mentioned), of hemoglobin (by Palmer's method [9]), and of p_H colorimetrically using phenolsulphonephthalein according to the method of Rowntree, Levy and Marriott,[10] and electrometrically by means of a hydrogen electrode previously described by De Eds and Hanzlik.[11] At the same time kymographic

9. Palmer: J. Biol. Chem. **33**:119 (Jan.) 1918.

10. Levy, R. L.; Rowntree, L. G., and Marriott, W. McK.: A Simple Method for Determining Variations in the Hydrogen-Ion Concentrations of the Blood, Arch. Int. Med. **16**:389 (Sept.) 1915.

11. De Eds, Floyd; and Hanzlik, P. J.: J. Biol. Chem. **60**:355, 1924.

records were made of changes in blood pressure, pulse, and respiration in the usual manner. Toward the end of each experiment, asphyxia was induced by clamping the trachea or attaching to it a long tube in order to increase the dead space. Then blood was drawn and the results of analysis were compared with those of the blood before asphyxia. In order to determine whether or not the changes in urea nitrogen and other constituents of the blood were characteristic for the intravenous injection of hexamethylenamin, control injections of hypertonic (10 per cent.) sodium chlorid and 6 per cent. acacia were made. The results of the experiments on dogs are summarized in Table 3.

TABLE 3.—*Changes in Urea Nitrogen and Other Constituents of the Blood of Dogs Infected Intravenously with Solutions of Hexamethylenamin, and Other Solutions as Controls*

Number and Weight (Kg.) of Dog	Doses of Agents Injected	Changes in Blood							
		p_H		Urea Nitrogen Mg. per 100 C.c.		Per Cent. Hemoglobin		Minutes	
		Before Injection	After Injection	Before Injection	After Injection	Before Injection	After Injection	After Injection	Remarks
3 (7.4)	40 per cent. hexamethylenamin, 1.3 gm. per kg.	7.3	7.0 6.6 6.8	10.53	11.49 11.05 11.0	85.1	79.0 75.2 73.0	15 31 54	Asphyxia
4 (9.88)	4.7 per cent. hexamethylenamin, 0.47 gm. per kg.	7.3	7.3 7.1 7.1 7.0	11.76	13.7	100	96.5 34.0 90.0 98.0	18 60 75 101	Asphyxia
5 (7.2)	40 per cent. hexamethylenamin, 1.36 gm. per kg.	7.3	7.1 7.2 6.8	8.5	9.76 10.93 11.1	100	92.6 100.0 80.4	24 39 69	Asphyxia
6 (23.5)	40 per cent. hexamethylenamin, 1.7 gm. per kg.	7.4	7.3 6.0 7.0	13.36	10.0 11.7 11.1	100	100.0	13 21 170	
7 (14.3)	40 per cent. hexamethylenamin, 1.4 gm. per kg.	7.3	7.3 7.4 6.9	8.0	7.5 10.0 8.14	100	93.4 100.0 100.0	17 77 84	Asphyxia
14 (6.6)	40 per cent. hexamethylenamin, 3.0 gm. per kg.	7.3	7.1 7.0 7.0	15.4	13.8 15.8 14.9	89.7	76.0 73.2 70.7	65 83 113	
16 (5.12)	40 per cent. hexamethylenamin, 4 gm. per kg.	7.0	6.9	14.6	14.6	100	77.5	24	
11 (19)	10 per cent. NaCl, 5.2 c.c per kg.	7.2	7.1	11.3	12.74	100	75.2	23	
19 (13.2)	6 per cent. acacia, 16 c.c. per kg.	7.15	7.1 7.0	8.5	9.3 11.1	100	97.0 96.0	31	

The results obtained indicate slight and negligible increases in the urea nitrogen content of the blood in the majority of the experiments, even in those in which asphyxia was induced and when the blood was truly acid, i. e., $p_H = 6.8$ to 6.9. In Experiments 1, 2 and 3, increases in urea nitrogen up to 30 per cent. occurred, despite dilutions of the blood due to the volume of fluid injected, and to dehydration of the tissues when hypertonic solutions were used. These increases in urea nitrogen may be due to a washing out of urea from the tissues. Since similar increases in nitrogen content of the blood occurred after injection of an isotonic solution (6 per cent. acacia in 0.9 per cent. sodium chlorid)

and also hypertonic (10 per cent.) sodium chlorid solution, the increases cannot be ascribed to hypertonicity, nor to ammonia derived from the hydrolysis of hexamethylenamin.

The shifting of the acid base equilibrium in these experiments constitutes a highly important and interesting phase of intravenous injections in general, and will not be discussed here. It has no bearing on the problem at hand.

The chief conclusion to be derived from the experiments on dogs for the purpose of this study is that the results as to the hydrolysis or decomposition of hexamethylenamin as indicated by changes in the urea nitrogen (indirectly ammonia) of blood are negative, even under the most favorable condition of asphyxia (acidity). They confirm the negative results on rabbits, and also those of the experiments in vitro with buffer serum mixtures.

CHANGES IN FORMIC ACID CONTENT OF THE BLOOD

Since formic acid is an oxidation product of formaldehyd, it was used as an index of the hydrolysis of hexamethylenamin in the blood.

TABLE 4.—*Formate Content of Blood After Intravenous Injection of Sodium Formate in a Dog Weighing 17.5 Kg.*

Time	Event	Formic Acid in Blood from	
		Jugular Vein	Femoral Artery
11:10	Blood sample (control)	None	None
11:19	Injection of sodium formate 0.28 gm. per kg.	.....	.....
11:25	Blood samples	2.85 mg. = 8.2 per cent. of injected amount	3.5 mg.
12:00	Blood samples	1.7 mg. = 0.5 per cent. of injected amount	2.25 mg.
1:00	Blood samples	0.78 mg. = 0.2 per cent. of injected amount	0.27 mg.

However, before proceeding to the results on formic acid, it should be indicated that the method used could detect formic acid after the injection of formate and of formaldehyd.

The formic acid was determined by the method [8] described in the foregoing. By this method formic acid added to blood in amounts as small as 0.1 mg. in 25 c.c. can be satisfactorily determined. As a further check on the method, analyses of the blood were made after injecting 50 c.c. of 10 per cent. sodium formate, equivalent to 3.4 gm. formic acid, into the femoral vein of a dog weighing 17.5 kg. The blood volume was taken as 9 per cent. of the body weight, or about 1,500 c.c., and, therefore, the concentration of formic acid was about 0.23 per cent.

Samples of 15 c.c. of blood were removed from the jugular vein and femoral artery, the theoretical amounts of formic acid injected being 34.5 mg.

The results in Table 4 indicate that the method was satisfactory for determining small quantities of formic acid, such as might be derived from the quantities of hexamethylenamin injected in the experiments to be described. They also show that formate disappears rapidly from the blood stream.

The results in Table 5 obtained on four dogs, injected intravenously with different concentrations of formaldehyd, indicate that detectable quantities of formic acid were found with concentrations of 1:7,000 and over of formaldehyd and that the percentage of formaldehyd oxidized to formic acid increased up to two hours. We may now briefly summarize the results of the experiments with hexamethylenamin.

TABLE 5.—*Percentage Conversion of Formaldehyd to Formic Acid in the Circulation of Dogs*

Dog	Weight, Kg.	Concentration of Formaldehyd Injected into Blood Stream	Per Cent. Converted to Formic Acid	Remarks
1	14	1:2,500	3.9 at end of 25 minutes 8.8 at end of 1 hour	Blood from heart
2	8.6	1:5,000	5.8 at end of 13 minutes 16.0 at end of 35 minutes 20.0 at end of 65 minutes	Blood from heart
3	9.5	1:7,000	1.6 at end of 2 hours and 15 minutes None at end of 2 hours and 40 minutes	Blood from jugular vein
4	8.6	1:15,000	None	Blood from femoral artery

Three dogs (Experiments 7, 9 and 10) were injected with a 10 per cent. solution of hexamethylenamin into the femoral vein after removal of control samples of blood. Then three samples were removed at approximately 30 minute intervals, and finally two samples in each case were taken after inducing asphyxia for the purpose of procuring the conditions most favorable for the liberation of formaldehyd. Electrometric and colorimetric determinations of the p_H of asphyxial bloods showed definite acidity in each case ($p_H = 6.81$ to 6.9). However, no formic acid was demonstrable in the bloods of all dogs before and after asphyxia. These negative results agreed with the relatively low, or almost negligible, hydrolysis of hexamethylenamin in vitro in the presence of 5 per cent. horse serum, which did not exceed 0.1 per cent. (Chart 3). The average blood volume of the dogs receiving injections of the drug was 900 c.c. and the amount of drug injected was 25 c.c. of a 10 per cent. solution, or 2.5 gm. If only 0.1 per cent. were hydrolyzed, the concentration of liberated formaldehyd would be about

1 : 300,000, providing the formaldehyd so liberated had been retained in the circulation. This is below (about one-tenth) the concentration of formaldehyd necessary for antisepsis. It is also probably smaller than the concentration of formaldehyd necessary for liberation of demonstrable formic acid in the blood. In other words, the results of the experiments on dogs and on hydrolysis in vitro are in essential agreement. They indicate that hydrolysis of hexamethylenamin in the blood is negligible and, therefore, that the concentration of liberated formaldehyd would be too small to be efficacious as an antiseptic.

6. EXCRETION OF FORMIC ACID IN URINE

The possibility still remains that formic acid might be formed slowly and appear in the circulation in amounts too small to permit estimation by the method used for blood. This might occur from slow decomposition of the hexamethylenamin itself, or of some combination of the liberated formaldehyd with or in the tissues, the combined formaldehyd requiring more time for oxidation to formic acid than was allowed in the acute experiments on animals. On the other hand, the absence of formate in hexamethylenamin urine collected until the excretion was completed (i. e., for from 2 to 3 days) would practically dispose of the occurrence of formaldehyd in the tissues, whether free as an intermediary product before conversion to formic acid or in some other oxidizable form. Combined formaldehyd in the blood and tissues would be of no more value as an antiseptic than hexamethylenamin itself. The matter was put to a test.

Determinations were made of the total excreted formate in three subjects, one of whom was a febrile patient, and two were healthy. The method used for estimation of formic acid in urine has been described in the foregoing.[8] Hexamethylenamin in doses varying from 1 to 5 gm. was administered by mouth, and total collections of urine (preserved with chloroform) made from the normal subjects, that is, until completion of the excretion of the hexamethylenamin and formaldehyd, as indicated by negative bromin water and phloroglucin tests, respectively. The excretions lasted for one and two days in the two healthy subjects. Only a partial collection of urine (during the first thirty-six hours after administration of hexamethylenamin) was made from the febrile patient. The formic acid was then estimated in aliquot portions of all the urines. The results that were obtained are briefly summarized in Table 6.

It is seen that the two healthy subjects excreted demonstrable though relatively small quantities of formic acid, amounting to 0.31 and 0.26 per cent. of the total hexamethylenamin administered. Subject 1 (F. D.), receiving the higher dose, excreted a greater absolute quantity and higher percentage of formic acid than Subject 2 (M. L. T.) who

received less than one-half the dose. The excretion, therefore, appeared to be influenced by the dosage, the absolute quantity of formic acid excreted being roughly proportional to the dosage. However, the excretion was independent of the diuresis, i. e., the total volume of urine excreted, since Subject 1 who excreted more formic acid had a smaller output of urine than Subject 2, whose output of urine was greater and excretion of formic acid smaller. The febrile patient excreted no demonstrable formic acid during the first thirty-six hours after administration of the drug, and despite a collection of 3,000 c.c. of urine, the highest output of all the subjects. The dosage of the drug also was the highest. Although the collection of urine from the patient was not complete, it extended over a considerable period (thirty-six hours) and formic acid was expected, but not found. During similar periods, formic acid was found in the urines of the

TABLE 6.—*Excretion of Formic Acid in Urine of Human Subjects Receiving Hexamethylenamin*

Subject	Body Weight, Kg.	Dose of Hexamethylenamin Administered, Gm.	Total Volume of Urine, C.c.	Total Formic Acid Excreted, Gm.	Per Cent. Formic Acid Excretion of Total Hexamethylenamin Administered
1 (F. D.) (healthy).....	55	2.5	1,500	0.0154	0.31
2 (M. L. T.) (healthy)...	77	1.0	1,750	0.0050	0.26
3 (H.) (febrile) (103 F.)..	99	5.0	3,000	None	None

two healthy subjects. The sensitivity of the method used for estimation of the formic acid was great enough, namely, 1:150,000, leaving no doubt that the urine was practically formic acid free. Hence, it appears that there was a complete oxidation of the formaldehyd or formic acid (to carbon dioxid and water) in this patient, as might occur from the high fever which was present. If this is true, antiseptic action from formaldehyd in infectious diseases (febrile usually) would hardly be expected. The time at disposal did not permit the study of other febrile patients. The results on the healthy subjects, in whom very little formic acid was liberated, indicated that excretion required no further study for the purpose of this investigation.

It was estimated from the results on Subject 1, in whom the greatest decomposition of hexamethylenamin occurred, that the concentration of formaldehyd in the blood could not have exceeded 1:500,000, and in Subject 2, not over 1:2,000,000. These figures were arrived at by assuming the blood volume to be about 9 per cent. of the body weight and the existence of all the formaldehyd (precursor of the formic acid) at one time. However, this probably was not the case, because the excretion lasted for one and two days, and, therefore, at any given

moment the concentration of formaldehyd in the blood was much less. Hence, it is clear that antiseptic qualities could not be conferred on the blood and tissues.

It is concluded that the administration of relatively small doses (1 and 2.5 gm.) of hexamethylenamin to healthy human subjects results in the excretion of small quantities (0.005 and 0.0154 gm.) of formic acid (as formate) in urine, amounting to about from 0.26 to 0.31 per cent. of the total drug administered, and indicating that the liberation of formaldehyd, as precursor of formic acid, in the tissues is too small for systemic antisepsis. In fever, formic acid may not be demonstrable, suggesting complete oxidation of the formaldehyd or formic acid, this condition, therefore, not favoring antisepsis.

COMMENT

The results have been sufficiently discussed throughout the text and need no further discussion here, except to indicate briefly their bearing on systemic antisepsis from hexamethylenamin. Studies of the urinary excretion showed that from 15 to 68 per cent. of the drug remained unaccounted for, indicating a marked loss of the drug in its passage through the body and agreeing with reports of other investigators. A part of this loss was accounted for by hydrolysis or decomposition of the drug in the alimentary tract (chiefly in the stomach). It appeared that decomposition in the tissues might account for the remainder. Tests of this were made, using direct and indirect evidence in vitro and in vivo. It was found that slight liberation of formaldehyd from the drug occurred in alkaline serum protein mixtures in vitro, the liberation being considerable in acid mixtures as would be expected. However, when blood was rendered definitely acid by asphyxia in animals (until death) injected with large doses of the drug, no increases in the production of decomposition or hydrolysis (ammonia, indicated by ureanitrogen, and formic acid) of hexamethylenamin were demonstrable. Small quantities of one of these products, namely, formic acid, were demonstrable in the urine of two healthy subjects receiving small doses of hexamethylenamin. This was probably due to the longer time allowed for collection of the formate in the excretion experiments than was allowed in the acute experiments on animals, in whose bloods the concentrations of the formic acid probably were too low for estimation. It appears therefore, that formaldehyd, as precursor of formic acid, is liberated in the tissues. However, the concentration at any given moment is much too low for antisepsis, amounting to about 1:500,000 at best, in a subject receiving 2.5 gm. of hexamethylenamin, or in other words, about one-twentieth of the concentration (1:25,000 or thereabouts) necessary for antisepsis. With higher doses the concentration might be higher, but at any given moment

and during the time of liberation the concentration would be much less than the highest estimated from the results in this paper. From all this, it follows that the alleged beneficial effects from hexamethylenamin systemically in various infectious diseases cannot be explained on the presence of adequate liberation of formaldehyd in the circulation and tissues. The drug itself it not antiseptic.

Taking the results of this study as a whole, two deductions appear justified. First, the incomplete and variable urinary excretion of hexamethylenamin, after administration by mouth, is accounted for by loss of the drug, that is, by hydrolysis or decomposition, in the alimentary tract, largely in virtue of gastric acidity, which is variable. The formaldehyd liberated in the stomach is not absorbed, as is well known. The decomposition of the drug in the tissues, as indicated by the excretion of formic acid, is very small, and accounts for only a very small fraction of the loss. Secondly, there is no good basis for the use of hexamethylenamin, orally or intravenously, as a systemic antiseptic in various infectious diseases and localized infections. Here again the excreted formic acid is too small in amount to account for the alleged benefits on the basis of the very low concentration of formaldehyd that might be liberated as an intermediary product to or precursor of the formic acid.

CONCLUSIONS

1. The total urinary excretion of hexamethylenamin administered to human subjects, in doses ranging from 1 to 5 gm., was found to be rather variable, ranging from 32 to 85 per cent., independently of dosage of the drug, diuresis and clinical condition.

2. Oral administration of sodium bicarbonate increased the excretion in two subjects (up to 100 per cent. in one), indicating that hydrolysis or decomposition of the drug occurs in the alimentary tract, chiefly in virtue of gastric acidity. In other tests, the excretion was virtually unchanged, indicating partial decomposition of hexamethylenamin by the tissues.

3. Decomposition, as indicated by liberation of free formaldehyd, in buffer phosphate mixtures in vitro was found to be a function of the hydrogen-ion concentration, that is, it increased with increases in true acidity. An appreciable liberation of formaldehyd was demonstrable on the alkaline side of neutrality.

4. In buffer mixtures containing serum protein the decomposition was much less as would be expected. At a p_H of 6.8 the maximum decomposition was about 0.1 per cent., and traces of formaldehyd were demonstrable at a p_H of 7.2 (vicinity of blood reaction).

5. In acute experiments intravenous injections of large doses of hexamethylenamin in animals did not yield evidence of decomposition as indicated by changes in or presence of decomposition products of the drug, namely, changes in ureanitrogen from the ammonia, and of formic acid (the oxidation product of formaldehyd) even under the most favorable conditions, that is, acidity (p_H 6.8) of blood induced by asphyxia.

6. Excretion experiments over longer periods in two healthy human subjects, receiving 1 and 2.5 gm. of hexamethylenamin orally, indicated that 0.26 and 0.31 per cent. respectively, of the drug were converted to formic acid in its passage through the body. In a febrile patient receiving a higher dose than the healthy subjects, namely 5 gm. of the drug, no formic acid appeared in the urine.

7. This means that either no formaldehyd (as precursor of formic acid) or only traces were liberated from hexamethylenamin in its passage through the blood and tissues.

8. Therefore, from all this, it appears that the hexamethylenamin (when given by mouth) unaccounted for by urinary excretion is lost or decomposed in the alimentary tract, chiefly in virtue of gastric acidity, and that there is no rational basis for the use of the drug, orally or intravenously, as a systemic antiseptic in the treatment of various infectious diseases and localized infections.

THE EFFECTS OF MITRAL STENOSIS, PULMONIC STENOSIS, AORTIC REGURGITATION AND HYPERTENSION ON THE ELECTROCARDIOGRAM*

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Just seven years ago Dr. Arlie V. Bock and I [1] presented before the American Society of Clinical Investigation a report entitled: "Electrocardiographic Evidence of Abnormal Ventricular Preponderance and of Auricular Hypertrophy." That report was based on the electrocardiograms of about 1,000 cases studied in the course of two and one-half years at the Massachusetts General Hospital. Our conclusions were that mitral stenosis and pulmonic stenosis do often affect the electrocardiogram to produce an abnormal swing of the electrical axis of the heart to the right, and that hypertension and aortic regurgitation often cause an abnormal swing of the axis to the left. We used the formula which Lewis [2] had suggested in 1914, simply the sum in tenths of a millivolt of the amplitudes of R in Lead I plus S in Lead III minus the sum of S in Lead I plus R in Lead III; that is $(R_1 + S_3) - (S_1 + R_3) =$ index. A value of this index more positive than + 20 we considered generally abnormal left axis deviation, and more negative than —15 generally abnormal right axis deviation. We had on our list eighty-two cases of the former and twenty-two of the latter. As the result of constant observations in the last seven years we have made but one change in this regard. We now feel that — 10 rather than — 15 should be the borderline of normal with respect to right axis deviation.

Shortly after the war, Carter, Richter and Greene [3] published the description of a method for determining the electrical axis of the heart by using Einthoven's equilateral triangle and measuring by angle, the right horizontal radius of the circle surrounding the triangle being the zero degree line. Radii above this give negative values and below positive. The normal limits we have taken at 0 and + 90 degrees, that

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* From the Massachusetts General Hospital.
* Read before the Association of American Physicians, Atlantic City, N. J., May 6, 1924.

1. White, P. D., and Bock, A. V.: Am. J. M. Sc. **156**:17 (July) 1918.
2. Lewis, T.: Heart **5**:398, 1914.
3. Carter, E. P.; Richter, C. P., and Greene, C. H.: Bull. Johns Hopkins Hosp. **30**:162 (June) 1919.

is, any angle falling in the right lower quadrant of the circle. We have used this method as well as the formula described previously in the analysis of all our cases. Usually, the two methods are in agreement but often they are not. Neither one is wholly reliable. Both are quite arbitrary but both seem to work out clinically fairly equally. In the case of significant right axis deviation, the angle method is in general perhaps more satisfactory than the index, while in the case of left axis deviation the index seems to fit better. We make a routine practice now of using both methods to measure abnormal axis deviation.

A few years ago Herrmann and Wilson [4] showed that there is little agreement between these methods of determining axis deviation and actual ventricular weights as determined at necropsy. Clinically, however, the influence of uncomplicated mitral stenosis, stenosis, hypertension, and aortic regurgitation on the electrocardiogram is a clear one. There are certain reasons for the discrepancies between the

TABLE 1.—*Cases with Electrocardiograms Showing Abnormal and Normal Electrical Axis Deviation*

	Right	Left	None
Number of cases	288	208	100
	Per Cent.	Per Cent.	Per Cent.
Mitral stenosis *	53	3(—)	14
Pulmonary stenosis *	7	0	0
Congenital dextrocardia *	2	0	0
Hypertension *	3(—)	30	3
Aortic regurgitation *	1(—)	20	4
Aortic stenosis *	0	2	0
Combined lesions and miscellaneous	34	45	79

* Uncomplicated.

electrocardiograms and actual ventricular weights. In the first place, as Burwell and I [5] pointed out a few years ago, the electrocardiogram at best is a crude measurement in this respect, as accurate as it may be in other regards. It is an arbitrary record made from three lead points in one plane, while of course the heart is a solid body and has three dimensions. Secondly, the shape of the person alters the anatomic axis considerably and also the electrical axis of the heart, and, of course, the actual ventricular weights do not take this into consideration.

Also it is quite probable that dilatation as well as hypertrophy plays its part in altering the electrocardiogram. Slight changes in intraventricular conduction rates undoubtedly play their part too. And finally it is probable, as has been already suggested, that there is an individual variation in the architecture of the auriculoventricular conduction tissue in different people. However these various factors may play their

4. Herrmann, G. R., and Wilson, F. N.: Heart **9**:91 (April) 1922.
5. White, P. D., and Burwell, C. S.: Tr. Am. Soc. Clin. Investigation, 1921, p. 20.

part, we do know that there is often significance clinically in electrocardiographic variations pointing to abnormal right or left axis deviation, as the following data will show. Cases showing intraventricular block are not included in this study.

In the course of nine years, over 4,000 persons, mostly cardiac patients, have been electrocardiographed at the Massachusetts General Hospital. Of these, 288 showed abnormal right axis deviation by either of the two methods I have described above. Fifty-three per cent. of these 288 cases had mitral stenosis, 7 per cent. had pulmonary stenosis and 2 per cent. had congenital dextrocardia, a total of 62 per cent. This may be compared with 100 unselected cases showing no abnormal axis deviation by either method, with 14 per cent. mitral stenosis and no cases of pulmonary stenosis or congenital dextrocardia.

During this interval of time there were 398 cases showing abnormal left axis deviation by either or both methods. Of these, 30 per cent. had hypertension of 170 mm. of mercury or over (systolic), 20 per cent. had aortic regurgitation, and 2 per cent. had aoritic stenosis without

TABLE 2.—*Relationship of Clinical Diagnosis to Electrical Axis Deviation*

	Normal	Mitral Stenosis*	Pulmonary Stenosis*	Hypertension*	Aortic Regurgitation*
Total number cases	100	100	20	100	50
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Abnormal right axis deviation	8	47	100	2	4
Abnormal left axis deviation	11	7	0	55	50
Normal axis deviation	81	46	0	43	46

* Uncomplicated.

obvious regurgitation, a total of 52 per cent. In the unselected group of 100 cases showing no abnormal axis deviation, there were only three cases with hypertension, four with aortic regurgitation, and none with aortic stenosis, a total of 7 per cent.

We have also made a study of the subject from the standpoint of clinical diagnosis. One hundred normal people of both sexes, and ranging in age from 8 months to 83 years, showed 8 per cent. with an index more minus than — 10, which 8 per cent. included the only two people who gave an angle greater than + 90 degrees. Three per cent. of these 100 normal persons showed an index greater than + 20. Two of these three cases had an angle of less than 0 degrees and there were eight additional persons with normal index but angle in the right upper quadrant.

COMMENT

For comparison, 100 cases of mitral stenosis uncomplicated by aortic regurgitation or hypertension were studied; 43 per cent. showed an index more negative than — 10 and an additional 8 per cent. had an index of just — 10. Not one of the 100 cases had an index as high

as + 20. Thirty-seven per cent. showed an angle greater than + 90 degrees, thirty-three of whom are already included in the forty-three showing an index more negative than — 10. Nine cases gave an angle of just 90 degrees. Seven cases showed a negative angle.

Incidentally, of the 57 cases showing normal rhythm of these 100 cases of mitral stenosis, forty-four (or 77 per cent.) had abnormally high P waves in Lead I (over one millimeter high), Lead II (over two millimeters high) or in both leads.

Twenty unselected cases of probable pulmonary stenosis in congenital heart disease all showed an index more minus than — 10 and all but three had angles greater than + 90 degrees, these three exceptions having angles of just 90 degrees. Incidentally in every case the P wave was abnormally prominent in Lead I (over one millimeter high), Lead II (over two millimeters high), or in both leads, indicating auricular hypertrophy, a fact not hitherto recorded so far as we know.

One hundred unselected cases of uncomplicated hypertension showed an index of over + 20 in 44 per cent. and a negative angle in 47 per cent., as compared with 3 per cent. and 10 per cent., respectively, in the group of 100 normal persons. Only two of the hypertensive cases gave an index more minus than — 10 and none had an angle as great as + 90 degrees.

Finally fifty unselected cases of aortic regurgitation uncomplicated by mitral stenosis or hypertension were studied, 38 per cent. of them showing an index of over + 20 and 40 per cent., a negative angle. But two cases had an index more minus than — 10 or an angle over + 90 degrees.

SUMMARY

Thus, in summarizing, it is evident that there is a definite relationship between abnormal right axis deviation by electrocardiogram and mitral stenosis and pulmonic stenosis, and between abnormal left axis deviation and aortic regurgitation and hypertension. Also the auricular complex of the electrocardiogram in Lead I, Lead II, or both, is almost always abnormally prominent in cases of pulmonary stenosis and of mitral stenosis.

A NEW LIVER FUNCTION TEST

THE ELIMINATION OF ROSE BENGAL WHEN INJECTED INTO THE CIRCULATION OF HUMAN SUBJECTS *

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AND
WILLIAM J. KERR, M.D.
SAN FRANCISCO

Various tests, which have been devised to estimate the functional capacity of the liver, have not proved to be of great clinical value. Most of these tests have been used in an attempt to study only one of the several well known functions of the liver. More recently some of the dyes have been employed as permeability tests for liver activity. Among the dyes advocated for this purpose have been methylene blue,[1] congo red,[2] indigo carmine,[3] and phenoltetrachlorphthalein. The last of these dyes has been most extensively studied. Rowntree, Hurwitz and Bloomfield [4] tried to estimate the permeability of the liver by the extraction of the dye from the feces after injection into the blood stream. This method was subject to many sources of error in the collection and extraction of feces and its use has largely been abandoned. The next step in advance was the modification of McNeil,[5] who used the duodenal tube and determined the amount of dye recoverable from the intestine. This method had obvious clinical limitations because of the difficulties in passing the duodenal tube and in collecting all the bile secreted in a given time. The test was further modified by Piersol and Bockus,[6] who probably achieved greater accuracy in estimating the appearance time of the dye in the duodenum after intravenous injection.

An approach to the problem from a different angle was made by Rosenthal [7] who devised a method of investigating the liver by inject-

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* From the Department of Medicine, University of California Medical School.

* Read before the Southern California Medical Association, Santa Barbara, Calif., April, 1924.

1. Cohn, H. M.: Klin. Wchnschr. **1**:2522 (Dec. 16) 1922.
2. Lepehne: München. med. Wchnschr. **69**:342 (March 10) 1922.
3. Einhorn, M., and Laporte, G. L.: New York M. J. **118**:350 (Sept. 19) 1923.
4. Rowntree, L. G.; Hurwitz, S. H., and Bloomfield, A. L.: Bull. Johns Hopkins Hosp. **24**:327, 1913.
5. McNeil, H. L.: J. Lab. & Clin. Med. **1**:822, 1915.
6. Piersol, G. M., and Bockus, H. L.: Observations on the Value of Phenoltetrachlorphthalein in Estimating Liver Function, Arch. Int. Med. **31**:623 (May) 1923.
7. Rosenthal, S. M.: J. Pharmacol. & Exper. Therap. **19**:385 (June) 1922; A New Method of Testing Liver Function with Phenoltetrachlorphthalein, J. A. M. A. **79**:2151 (Dec. 23) 1922.

ing the dye into the blood stream and observing the concentration of the dye remaining after certain intervals of time. Rosenfield and Schneiders [8] have used an almost identical method, with some variations in technic. The results obtained by the latter two investigators, Rosenthal [7] and Rosenfield and Schneiders,[8] are in direct accord with our findings with rose bengal, since they show that phenoltetrachlorphthalein and rose bengal remain in the circulation for a longer period of time in cases where there is evidence of liver disease. This retardation in the elimination has been taken as evidence of liver injury.

In a previous communication one of us [9] discussed the elimination of rose bengal from the circulation after its injection into the blood stream of dogs, and the variation of the rate of its elimination after experimentally produced liver injury. In this paper we will point out the close similarity between the rate of elimination of the dye after injection into the human circulation and its elimination after injection into the circulation of dogs, and will note the modification of this rate of elimination in cases of definite liver disease.

METHOD

The dye used in these experiments, as before mentioned, is rose bengal, diiodotetrachlorfluorescence of the triphenylmenthane series. As stated in our previous paper it was found to be entirely eliminated by the liver after injection into the circulation, the urine remaining free from the dye in animals. No evidence of toxicity was noted from dosages up to 100 mg. of the dye for a dog weighing 18 kg. We felt encouraged to try the same method on human subjects, using smaller doses. We found, however, in one subject that the dye caused a rise in temperature; in another a rise in temperature and a slight chill occurred. The latter (Case 16) had a marked, and the former, a moderate retention of the dye in the blood stream. In all cases, including those with marked biliary obstruction, the urine remained free from the dye, with the exception of Case 14, in which a most marked retention of the dye in the blood stream was observed. This patient suffered from carcinoma of the common bile duct. It is of interest to note that the ascitic fluid of Case 21, withdrawn soon after the injection of rose bengal into the blood stream, showed traces of the dye, but they were too small to estimate. Further work on the physiologic chemistry and the extremely interesting behavior of the

8. Rosenfield, H. H., and Schneiders, E. F.: Improved Phenoltetrachlorphthalein Test for Liver Function, J. A. M. A. **80**:743 (March 17) 1923.

9. Delprat, G. D., Jr.: Studies on Liver Function; Rose Bengal Elimination from the Blood, as Influenced by Liver Injury, Arch. Int. Med. **32**:401 (Sept.) 1923.

dye has been undertaken by Dr. C. L. A. Schmidt, in the Department of Biochemistry, University of California Medical School.

Another characteristic of the dye is its photodynamic activity which, as has been demonstrated by Schmidt and Norman,[10] is more marked in rose bengal than in the other members of the triphenylmethane series. This property of the dye is of practical moment in its application in this work, since in our earlier human subjects we experienced considerable difficulty with hemolysis. To reduce this factor to a minimum the technic of the test has been modified, in that the blood is collected and kept before centrifugating in a subdued light and centrifugated as soon as possible after collection. As a precautionary measure, the patient is kept in a darkened room for an hour following the injection of the dye, although it is improbable that sunlight would have a markedly deleterious effect on a patient into whom the dye had been injected, since the injection of 100 mg. into a normal dog weighing 9 kg. caused no discernible effect after the dog had been exposed to sunlight for several days. This point was not emphasized in our previous paper, because the red cells of the dog appear to be more resistant to hemolysis than human red cells.

The technic described further on seems to have certain advantages: in the first place the patient is subjected to a single puncture of the vein; secondly, the actual time of operation on the patient is less than ten minutes and the final mathematical result of the test is available in sixty minutes from the time of its commencement. The dye is easy to obtain in bulk, can be sterilized without chemical change and is not expensive.

TECHNIC

The technic of injection of the dye and the collection of blood samples is exactly the same as that described in our previous paper. A vein in the cubital fossa is selected and a sample of blood is withdrawn with a syringe and discharged into a graduated centrifuge tube containing 2 c.c. of a 2 per cent. solution of potassium oxalate. Without the needle being removed from the vein, either 100 or 150 mg. of the dye, in a sterile 1 per cent. physiologic sodium chlorid solution, is injected and the needle washed out by a further injection of 5 or 10 c.c. of the solution. The needle is left in the vein, and at exactly two minutes after the injection of the dye a sample of blood (10 c.c.) is withdrawn from the needle still in situ into a fresh syringe, and discharged into another graduated centrifuge tube containing 2 c.c. of oxalate solution. The needle is again washed by injecting 5 or 10 c.c. of physiologic

10. Schmidt, C. L. A., and Norman, G. F.: Protection Afforded to Red Cells Against Hemolysis by Eosin, Jour. Infect. Dis. **27**:40 (July) 1920; J. General Physiol. **6**:681 (July) 1922.

sodium chlorid solution, which maneuver prevents the clotting of blood in the needle. At four and eight minutes, respectively, from the time of injection samples of blood are withdrawn and collected in an identical manner. The needle is then withdrawn from the vein in the arm and the patient is told to remain in the darkened room for an hour. It is our practice to advise the patient that the appearance of the dye in the stools will impart to them a distinct red color, since the unexpected appearance of the red stools may cause considerable alarm.

As soon as possible after collection, the blood samples are centrifugated at a speed of 2,000 revolutions per minute for thirty minutes. The percentage of cells and plasma in each tube is then carefully noted. Three cubic centimeters of plasma from the samples of blood taken at two, four and eight minutes respectively after the time of injection, are then diluted in separate tubes with an equal volume of physiologic sodium chlorid solution, and the color of the dye in these solutions is compared in a Helling colorimeter, with a standard solution containing 5 c.c. of plasma from the "control tube," that is, the sample of blood withdrawn from the vein before the injection of the dye, and 5 c.c. of an 0.0075 per cent. solution of rose bengal. It is evident that an allowance must be made for the fact that the plasma in the graduated centrifuge tube contains 2 c.c. of potassium oxalate solution, and that some correction of the colorimeter reading must be made to allow for this dilution.[11]

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11. For example: after centrifugating for thirty minutes the graduated centrifuge tube contained 4.3 c.c. of cells and 7.0 c.c. of supernatant fluid. The colorimeter reading of the plasma was 25 per cent. of the standard (0.0075 per cent. solution). If this plasma did not contain the 2 c.c. of oxalate the reading would have been twenty-five times 7.0 divided by 5.0. But the dye which is found in the supernatant 5.0 c.c. of plasma was, before centrifugating in the total 9.3 c.c. of blood. Hence the correction of the colorimeter reading, giving the correct percentage of the dye in the blood stream, is represented by $\frac{25 \times 7.0 \times 5.0}{5.0 \quad 9.3}$ (which is 18.8) per cent. of the 0.0075 per cent. solution of rose bengal. After these corrections have been made we obtain what we have termed "corrected colorimeter readings," such as are found in the charts.

Having obtained the concentration of the dye in the blood sample withdrawn two minutes after the injection of the dye and knowing the total amount of the dye injected into the circulation, it is a matter of simple proportion to calculate the blood volume of the person.

To continue the given example: two minutes after injection of 100 mg. of dye into the circulation the concentration of the dye in the blood was 18.8 per cent. of an 0.0075 per cent. solution of the dye, which is 0.00141 per cent. That is, 100 c.c. of blood contains 1.41 mg. of dye. Therefore, the blood volume of the person, into which this injection was made is $\frac{100 \times 100}{1.41}$ c.c., or 7,092 c.c.

This can readily be reduced for convenience in calculation to the following formula, which differs somewhat from the formula of our previous paper:

$$\text{Blood volume} = \frac{\text{Milligrams of dye injected} \times \text{volume of blood withdrawn} \times 10^9}{\text{Uncorrected colorimeter reading} \times 0.0075 \times \text{centrifuge tube contents less cell volume}}$$

Since we have injected a uniform amount of dye into persons of different weight and therefore of different blood volumes, it follows that the concentration of the dye in the blood samples obtained two minutes after injection should show considerable variation, and that it would be impossible to compare the corrected colorimeter readings of different persons with one another unless these readings were reduced to some arbitrary standard blood volume. The standard blood volume selected arbitrarily and purely for convenience for the purpose of this paper is 7,000 c.c. Thus in the foregoing the corrected colorimetric reading of the "two minute sample" of blood is 18.8 per cent. of an 0.0075 per cent. solution of the dye, from which the blood volume of 7,070 c.c. can be calculated. If now we reduce this to our standard blood volume of 7,000 c.c. it is evident that it is necessary to multiply the reading 18.8 by 7,070 and divide the result by 7,000 which gives 19.1 per cent. of an 0.0075 per cent. solution. This figure (19.1) is necessarily a mathematical constant since it expresses the concentration of a fixed amount of dye (100 mg.) injected into a fixed volume (7,000 c.c.).

Occasionally in large persons we have injected 150 mg. of dye instead of 100 mg., in which case we multiply the reading, which has been reduced to the standard blood volume of 7,000 c.c., by 100 and divide the result by 150, thus standardizing the dosage as well as the blood volume. After these corrections have been made it is possible to compare the rates of elimination of the dye from the circulation of different persons one with the other. Charts 1, 2 and 3 show curves representing the rate of elimination of the dye from the blood streams of the patients whom we have studied. Since the liver is presumably acting on a progressively decreasing amount of dye remaining in the circulation, it would seem correct to use as the ordinates of the curves the logarithms of the different concentrations of the dye.

Chart I shows the logarithmic curves of the concentration of the dye four and eight minutes after injection of 100 mg. of rose bengal (when computed on the basis of a standard blood volume of 7,000 c.c.), in a series of twenty-four cases clinically free from liver disease selected from the medical wards of the University of California Hospital. In these cases the average concentration of the dye four minutes after injection was 0.00116 per cent., varying between 0.00137 per cent. and 0.00060 per cent.; and eight minutes after injection the average concentration was 0.00085 per cent., varying between the limits of 0.00102 per cent. and 0.00061 per cent.

Chart 2 shows the logarithmic curve of a series of thirteen cases with clinical evidence of liver disease. In this group the average concentration of the dye in the blood four minutes after injection was

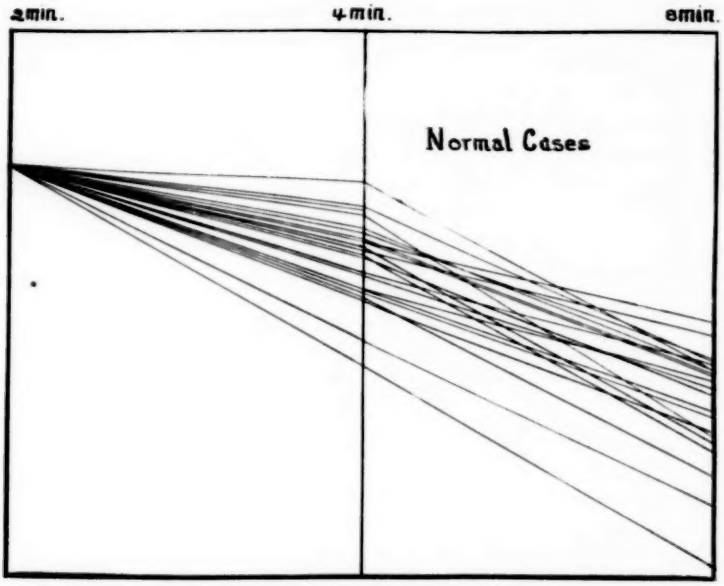

Fig. 1.—Logarithmic curves of the concentration of rose bengal in the circulation of normal persons after the intravenous injection of 100 mg. of the dye. Colorimetric readings have been corrected to an arbitrary blood volume of 7,000 c.c. for comparison.

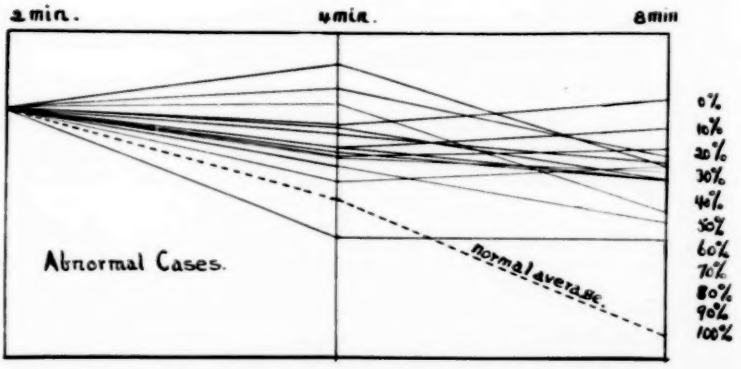

Fig. 2.—Curves obtained on a group of patients with liver disease. Percentages at the right of chart express the suggested function of the liver.

0.00136 per cent., varying between 0.00160 and 0.00116 per cent., and eight minutes after injection the average concentration was 0.00124 per cent., varying between 0.00144 and 0.00116 per cent. It can be seen that the average concentration of the dye in the blood four and eight minutes after injection in the group with liver disease is considerably higher than in the normal group, and that the lowest concentration reached at four and eight minutes in the abnormal group is considerably above the highest variation in the normal group. The group with liver disease is composed of the following cases:

ABSTRACT OF ABNORMAL CASES

CASE 14.—Carcinoma of head of the pancreas, complete biliary obstruction, no ascites.

CASE 15.—Cirrhosis of the liver, end stage of Banti's disease, marked ascites, confirmed at operation.

CASE 16.—Syphilis of the liver, jaundice, ascites.

CASE 17.—Carcinoma of the head of the pancreas, with complete obstruction, confirmed at operation.

CASE 18.—Cirrhosis of the liver, jaundice, ambulatory.

CASE 19.—Cirrhosis of the liver, marked ascites.

CASE 21.—Cirrhosis of the liver, marked ascites, jaundice.

CASE 30.—Enlarged liver, mediastinal tumor, question of metastatic malignancy of the liver.

CASE 31.—Advanced cirrhosis, von Jaksch disease, confirmed by necropsy.

CASE 33.—Cirrhosis with marked ascites.

CASE 39.—Moderately advanced cirrhosis of the liver with ascites.

CASE 20.—Postoperative cholecystitis and cholelithiasis. Probable cirrhosis.

CASE 22.—Cancer of rectum, extensive liver metastases.

This group includes persons with complete biliary obstruction, where no elimination of the dye through the liver could be expected.

Chart 3 includes a group of seven patients who have a history of probable chronic cholecystitis. This group includes men and women who were apparently in good health and ambulatory, but who could not be classified as normal because of the possibility of liver disease. It is not surprising that persons appear in this group who show curves which may well be classified as normal on the basis of the test that has been outlined in the foregoing. These cases are numbered 4, 13, 34, 35, 36, 37 and 38. The curve in Case 4 lies just above the upper limit of variation of the normal group but can hardly be considered as definitely abnormal. The border-line group includes:

CASE 4.—Chronic passive congestion.

CASE 13.—Chronic interstitial nephritis and cardiac failure, with general anasarca, chronic passive congestion of the liver.

CASE 34.—Chronic cholecystitis.

CASE 35.—Chronic cholecystitis.

CASE 36.—Cirrhosis of the liver (seen three years before at operation), patient ambulatory and apparently well.

CASE 37.—Chronic cholecystitis and cholelithiasis.

CASE 38.—Chronic cholecystitis.

This last group of cases illustrates the probability that a considerable degree of liver damage must exist before any variation from a group of normal persons can be determined; and that although a pathologic condition of the liver tissue may be determined microscopically, that enough normal parenchyma exists to carry on the various functions of the organ. This same limitation is seen in the permeability test of the kidney with phenolsulphonephthalein.

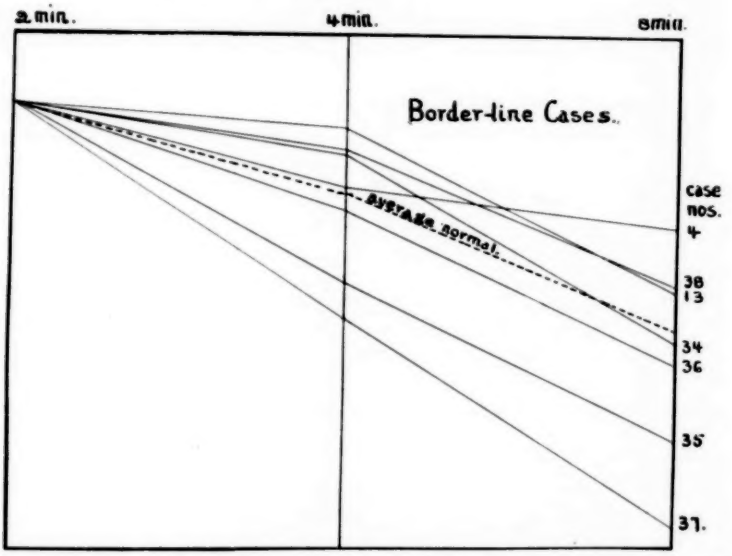

Fig. 3.—Curves obtained on a group of border-line cases with possible liver disease.

CONCLUSIONS

1. In normal persons, following the injection of a given amount of rose bengal into the circulation, the concentration of the dye computed under standard conditions of dosage and blood volume does not exceed certain limits at four and eight minutes after the time of injection.

2. In abnormal cases, where evidence of liver disease can be demonstrated clinically, the concentration of the dye in the circulation computed under standard conditions of dosage and blood volume, at four and eight minutes from the time of injection, exceeds the limits of concentration of the normal cases. Complete biliary obstruction, which

can be determined by other clinical tests, causes a retention of the dye in the blood stream.

3. When it can be shown that the rate of elimination of rose bengal from the circulation, after injection, is reduced, or in other words, when the concentration of the dye at four and eight minutes after injection exceeds the normal limits, a certain degree of liver dysfunction may be assumed to exist in the absence of complete biliary obstruction.

4. A rough estimate can be formed from the concentration of the dye remaining in the circulation at four and eight minutes after injection of the extent of the liver disease.

STUDIES IN THE DIASTATIC ACTIVITY OF THE BLOOD, WITH A CONSIDERATION OF ITS VALUE IN CLINICAL DIAGNOSIS *

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In 1917, Myers and Killian [1] published a simple, delicate and accurate method for estimating the diastatic activity of the blood. Briefly, the method consists in allowing a known quantity of blood (2 c.c.) to act on a known quantity of pure soluble starch (1 c.c. of a 1 per cent. solution = 10 mg.) for a definite length of time (fifteen minutes) in a water bath at 40 C. The glucose thus produced by the blood diastase is then determined by the Myers and Bailey method.[2] A control is run in a second tube to determine the original glucose contained in the 2 c.c. of blood, which is deducted from the total glucose found in the first tube. The results are recorded in terms of the percentage of the starch (10 mg.) transformed to reducing sugar (calculated as glucose) by the 2 c.c. of blood employed. Thus, by a "diastatic activity of 15" is meant that 15 per cent. of the 10 mg. of starch was converted into glucose by the diastase in the 2 c.c. of blood used.

In introducing their method, Myers and Killian state that, concerning the question of blood diastase in human diabetes, "Foster, Bainbridge and Beddard, Schlesiner, Wynhausen, Ghedini, and Stocks, were unable to come to any conclusions, though Loewi regarded the diastase of considerable importance, and Moeckel and Rost state, apparently from meager data, that the diastatic activity of the blood in human diabetes is almosts always higher than normal." In a study of blood diastase, on a series of normal and miscellaneous hospital cases, Myers and Killian found that in normal blood the diastatic activity, as estimated by their method, varied from 15 to 25. In a series of thirteen cases of diabetes they found the diastase much increased, the figures varying from 39 to 74. In eleven of 23 cases of nephritis they found the diastase to range from 30 to 52. The fact that these cases, in which a high blood diastase was noted, also showed a hyperglycemia, suggested to the authors the idea "that the increased diastatic activity in both diabetes and nephritis (as shown by the analyses of the blood) may be

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* From the Medical Clinic of the Peter Bent Brigham Hospital, Boston.

1. Myers, V. C., and Killian, J. A.: Studies on Animal Diastases, J. Biol. Chem. **29**:179 (March) 1917.

2. Myers, V. C., and Bailey, C. V.: The Lewis and Benedict Method for the Estimation of Blood Sugar with Some Observations Obtained in Disease, J. Biol. Chem. **24**:147 (Feb.) 1916.

the important factor in the production of the hyperglycemia in these conditions." As a result of their studies, Myers and Killian concluded that "a fall in the blood diastase would appear to afford a more reliable guide to the efficacy of the dietetic treatment in diabetes than either the blood sugar or urine sugar. Furthermore, an increase in blood diastase may constitute a very early sign of impending diabetes."

DeNiord and Schreiner [3] failed to confirm the findings of Myers and Killian. Using the Myers and Killian method for blood diastase estimation, DeNiord and Schreiner reported their observations on six cases of diabetes, all of which showed a low or normal blood diastase. Since these cases were syphilitic, and the results so opposed to the work of Myers and Killian, DeNiord and Schreiner suggested that a low diastatic activity in diabetes was possibly indicative of an associated syphilitic infection.

Block,[4] after summarizing the literature on the subject, arrived at the conclusion that the determination of the diastatic activity of the blood was of but limited clinical significance; that the normal variations were exceedingly great, and that unless the diastatic activity of the blood was increased to a value of 100 [5] or more it could not be regarded of diagnostic import. However, in Block's opinion, an increase in blood diastase to 100 or more is indicative of pancreatic disease. Block included in this connection, diseases of the neighboring organs affecting the pancreas secondarily, such as a duodenal ulcer perforating into the pancreas or obstructing the pancreatic duct; but he did not include diabetes. In the latter instance he found the reports conflicting, and his own observations did not warrant him to regard the blood diastase of any significance in this disease.

The present study was undertaken in order to establish the clinical value of blood diastase determinations, made according to the method of Myers and Killian, in diabetes, nephritis and diseases of the pancreas. For this purpose the following plan of investigation was carried out.

3. DeNiord, H. H., and Schreiner, B. F.: Diastatic Activity of the Blood in Cancer, Syphilis and Diabetes, Arch. Int. Med. **23**:484 (April) 1919.

4. Block, W.: Die praktische Verwertbarkeit der Amylase- (Diastase-) Bestimmung in Blut und Urin für die Diagnostik der verschiedensten pathologischen Zustande, Ztschr. f. klin. Med. **93**:381, 1922.

5. Block does not state clearly what unit of diastatic activity he used; but from the text one may infer that he employed Wohlgemuth's (Biochem. ztschr. **9**:1 [Jan.] 1908) colorimetric method, which is based on an iodin starch reaction, and the figure 100 refers to the number of cubic centimeters of 1 per cent. starch solution that could be converted into dextrin by 1 c.c. of blood serum in twenty-four hours at 40 C. From a comparative study of the results on Block's normal subjects, obtained by this method, and my normal figures obtained by the Myers and Killian method, I regard the value of 100 by Wohlgemuth's method to be equivalent to about 50 or 60 by the Myers and Killian method.

METHOD OF INVESTIGATION

1. The diastatic activity of normal human blood was determined in thirty-five cases in order to establish a normal standard. These estimations included observations on the effect of food and time of day on blood diastase in normal subjects.

2. The diastatic activity of diabetic blood was determined in twenty-five cases. Observations were also obtained on the immediate effect of food and insulin on the blood diastase in diabetes.

3. The diastatic activity of dog's blood was determined in four normal and two depancreatized dogs.

4. The diastatic activity of human blood was determined in one case of carcinoma of the pancreas.

5. The diastatic activity of human blood was determined in five cases of nephritis.

The results of this study are tabulated.

DIASTASE IN NORMAL HUMAN BLOOD

For the purpose of establishing an accurate normal standard two groups of cases were studied. One group consisted of thirteen normal

TABLE 1.—*The Diastatic Activity of Normal Human Blood* *

Case		Diagnosis	Blood Sugar	Units of Diastatic Activtiy	Remarks
1	R	Normal	0.113	10	
2	I	Normal	0.140	10	
3	C	Normal	0.130	11	
4	S	Normal	0.140	6	
5	A	Normal	0.112	7	
6	Student	Normal	0.091	19	
7	Student	Normal	0.090	12	
8	Student	Normal	0.099	16	
9	C	Normal	0.119	8	
10	K	Normal	0.123	11	
11	D	Normal	0.100	6	
12	S	Normal	0.109	12	
13	D	Normal	0.102	8	
14	C	Duodenal ulcer	0.114	14	
15	B	Duodenal ulcer	0.117	12	
16	R	Duodenal ulcer	0.091	9	
17	S	Duodenal ulcer	0.109	17	
18	Y	Duodenal ulcer	0.099	9	
19	B	Rheumatic pericarditis	0.113	14	Convalescent. Practically well
20	J	Typhoid	0.107	24	Convalescent. Practically well
21	B	Duodenal ulcer	0.100	11	
22	F	Chronic diarrhea	0.113	15	
23	L	Duodenal ulcer	0.125	18	Diagnosis questionable
24	H	Duodenal ulcer	0.104	23	
25	L	Duodenal ulcer	0.099	13	Diagnosis proved at operation and at necropsy
26	N	Pneumonia	0.130	21	Convalescent. Practically well
27	G	Medical observation	0.099	16	
28	P	Neurosis	0.094	14	
29	Ch	Respiratory infection	0.107	13	Convalescent. Practically well
30	Ch	Pulmonary tuberculosis	0.107	18	Inactive process
31	Sh	Asthma, bronchial	0.084	16	No recent attacks
32	G	Pelvic tumor	0.097	15	Proved to be carcinoma
33	X	Coma	0.174	6	Due to illuminating gas poisoning
34	X	Acute diarrhea	0.192	18	Young child
35	De	Medical observation	0.124	2	

* Cases 14 to 35 represent a group of miscellaneous hospital cases, which for the purpose of this investigation may be regarded as essentially normal.

persons composed of students and members of the hospital staff. The other group consisted of twenty-two miscellaneous hospital patients, of whom all but two showed a normal blood sugar. The results are recorded in Table 1.

As can be seen from examining the figures in this table, the diastatic activity in normal blood may vary between 2 and 24. Myers and Killian,[6] give from 15 to 25 as the normal limits. In repeating blood diastase estimations on the same person at intervals of a week or more, I obtained much more constant figures, five units being the highest variation noted. It appears, therefore, that, although in different persons blood diastase may vary between wide limits, in the same subject the variations are much less marked.

TABLE 2.—*The Effect of Food on the Diastatic Activity of Normal Human Blood*

Time	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6	
	Blood Sugar	Units of Diastatic Activity	Blood Sugar	Units of Diastatic Activity	Blood Sugar	Units of Diastatic Activity	Blood Sugar	Units of Diastatic Activity	Blood Sugar	Units of Diastatic Activity	Blood Sugar	Units of Diastatic Activity
Fasting.....	0.102	8	0.100	6	0.117	11	0.107	24	0.125	18	0.130	21
½ hour after meal.....	0.137	9	0.108	4	.....	..	.....	..	.....	..	.....	..
1 hour after meal.....	0.136	4	0.108	6	.....	..	.....	..	.....	..	.....	..
1½ hours after meal.....	0.113	8	0.124	2	0.122	12	0.103	23	0.140	22	0.115	20
2 hours after meal.....	0.113	7	.....	..	.....	..	.....	..	.....	..	.....	..

Table 2 gives the results of experiments which were made to show the effect of food on the diastatic activity of the blood in normal subjects. The figures obtained in these experiments seem to indicate that normal blood diastase is not affected by food or time of day. Those findings are in accord with the observations of Carlson and Luckhardt,[7] who performed similar experiments on animals.[8]

6. Myers, V. C.: Practical Chemical Analysis of Blood, Ed. 2, St. Louis, C. V. Mosby Co., 1924, p. 84.

7. Carlson, A. J., and Luckhardt, A. B.: On the Diastase in the Blood and the Body Fluids, Am. J. Physiol. **23**:148 (Dec.) 1908.

8. Carlson and Luckhardt studied blood diastase in a variety of animals, including dogs, cats, rabbits, pigs, sheep, goats and pigeons. They measured the concentration of the diastase in the serum "by (1) the rate of clearing and (2) by the rate of complete disappearance of erythrodextrin in the starch solution. The starch solution and serum or lymph were usually mixed in the proportion of from 5 to 0.1 c.c. and kept in thermostat at 38 C." The unit of diastatic activity used by these authors is not clearly defined. The concentration of the blood diastase was recorded as "equal," "greater" or "less" than some assumed standard, thus indicating respectively an equal, shorter, or longer time required to clear the starch solution at the given temperature (38 C.) and at the given proportion of starch serum mixture (5 to 1 c.c.).

BLOOD DIASTASE IN DIABETES

Table 3 shows the results obtained in a series of twenty-five cases of diabetes. They were all typical cases with the exception of one patient (Case 25) who came in to the hospital with symptoms of acromegaly and pituitary disease. An examination of the results recorded in this table reveals the fact that, with the exception of the

TABLE 3.—*The Diastatic Activity of the Blood in Diabetes*

Case		Blood Sugar	Units of Diastatic Activity	Remarks
1	C	0.149	12*	Urine sugar + (mild case)
2	O.D.	0.214	12	Urine sugar ++
3	L	0.256	11*	Urine sugar ++
4	R	0.380	15*	Urine sugar +++
5	B	0.310	14*	Urine sugar ++++
6	F	0.295	14*	Urine sugar +
7	R	0.245	3*	Urine sugar + (trace)
8	W	0.185	10*	Urine sugar ++
9	P	0.221	18	Urine sugar +++
10	R	0.425	19	Urine sugar ++++
11	McL	0.136	9*	Urine sugar +
12	Sh	0.250	17*	Urine sugar 1.8 per cent.
13	W	0.240	6*	Urine sugar 6 per cent.
14	K	0.390	25*	Urine sugar 1.4 per cent.
15	R	0.250	26*	Urine sugar ++
16	H	0.221	16*	
17	C	0.367	18*	Under treatment with insulin
18	T	0.198	18*	Only traces of sugar in urine
19	X	0.353	14	Urine sugar 7 per cent.
20	G	0.362	10*	Urine sugar 6 per cent.
	G	0.375	10.6*	Urine sugar 5 per cent.
21	S	0.263	10*	Urine sugar +
22	B	0.540	12	Urine sugar +++
	B	0.222	17	Urine sugar neg.
23	Bl	0.185	23	Urine sugar ++
24	P	0.340	15	Urine sugar ++++
25	R	0.230	32	Evidence of pituitary tumor with acromegaly symptoms. Urine sugar ++

* Blood drawn during fasting state. Others were examined in from 1 to 2 hours after meals.

TABLE 4.—*The Effect of Food on the Diastatic Activity of the Blood in Diabetes*

Time	Case 1		Case 2	
	Blood Sugar	Units of Diastatic Activity	Blood Sugar	Units of Diastatic Activity
Fasting	0.363	10	0.300	14
One-half hour after meal	0.425	9.4	.....	..
1 hour after meal	0.483	11.4	.....	..
1½ hours after meal	0.487	7	0.540	12

one typical case, the diabetics in this group yielded figures for blood diastase essentially like those obtained in our series of normal persons. Syphilis was excluded as a factor, since the histories justified such exclusion in all cases but one, and the Wassermann reaction was negative in every instance.

Table 4 records the results of experiments which were made to show the effect of food on the blood diastase in diabetes. The results obtained in this study are much like those seen in similar experiments

on normal persons. It is interesting to note that although the blood sugar rose considerably after the patient had taken food, the blood diastase remained either unaffected or was even lowered. These results speak against any relationship between hyperglycemia and increased diastatic activity of the blood.

EFFECT OF INSULIN

Table 5 records the results of experiments made to demonstrate the effect of insulin on the diastatic activity of the blood in diabetes. It will be seen from an examination of these figures [1] that although the blood sugar showed a marked and steady reduction in each instance,

TABLE 5.—*The Effect of Insulin on the Diastatic Activity of the Blood in Human Diabetes*

	Case 1			Case 2	
	Blood Sugar	Units of Diastatic Activity		Blood Sugar	Units of Diastatic Activity
Dec. 17, 1923. Six weeks before the insulin experiment, Case 1 was examined in the Outdoor Department with these findings.....	0.200	11			
Experiment: Jan. 29, 1924:					
Before insulin	0.540	10	Before insulin	0.353	14
½ hour after 10 units of insulin...	0.465	16	20 units given	.....	..
1 hour after 10 units of insulin....	0.395	17	1 hour later	0.166	19
1½ hours after 10 units of insulin..	0.387	17	1½ hours later	0.102	14
2 hours after 10 units of insulin....	0.353	13	.....	.....	..
2½ hours after 10 units of insulin..	0.342	12	2½ hours later	0.111	14
Feb. 6. After receiving one week's careful dietary treatment in the ward, with a sugar free urine, Case 1, on reexamination showed.	0.220	17			

there was no corresponding change in the blood diastase. On the whole, the variations noted were not unlike those seen in similar serial determinations in both normal and diabetic subjects who had not received insulin; and the results suggest that insulin is without significant influence on the diastatic activity of the blood in diabetes. Myers,[6] on the other hand, reported a similar experiment in one case, noting a slight reduction in the blood diastase after insulin.

BLOOD DIASTASE IN NORMAL AND IN DEPANCREATIZED DOGS

The blood diastase in four normal dogs was estimated by the Myers and Killian method. The pancreas was then removed in two of these dogs, and their blood again examined for the diastatic activity. The results of these experiments are recorded in Table 6. As may be seen from an examination of these results, the diastatic activity of normal dog's blood is from three to six times as high as the diastatic activity of normal human blood. The blood diastase estimations made in the

depancreatized dogs yielded figures slightly lower than those obtained in the same dogs before the operation or in the other normal dogs.

These findings correspond with the observations of Carlson and Luckhardt,[7] who performed similar experiments on two cats. Carlson and Luckhardt were able to keep these animals alive for eight and eleven days respectively. Frequent diastase determinations on these animals showed, in one animal, a slight reduction in the diastatic activity during

TABLE 6.—*The Diastatic Activity of the Blood in Normal and in Depancreatized Dogs*

Experiments	Blood Sugar	Units of Diastatic Activity
Dog 1. Normal	0.097	73
Dog 2. Normal	0.107	87
Dog 3. Normal	0.107	77
Dog 4. Normal	0.116	77
Dog 1. Immediately after total extirpation of pancreas	0.222	65
Dog 2. Immediately after total extirpation of pancreas	0.200	72
Dog 2. Five hours after operation. Dog has completely recovered.	0.193	67
Dog 1. Two days after operation. Dog apparently completely recovered from operation and is running around like a normal dog	0.346	55

the first five days after the operation, with a return to normal on the eighth day; in the other animal the diastatic activity remained normal till its death on the eleventh day after the operation. However, Myers and Killian report a decided increase in the blood diastase in one depancreatized dog, and Milne and Peters [9] report increases in the blood serum diastase in a large series of dogs after complete or partial removal of the pancreas.[10]

BLOOD DIASTASE IN DISEASE OF THE PANCREAS

It has been seen that pancreatectomy did not immediately affect the concentration of the blood diastase to any extent in two dogs. To

TABLE 7.—*The Diastatic Activity of the Blood in Pancreatic Disease*

Experiments	Blood Sugar	Units of Diastatic Activity
1. Human: Carcinoma of head of pancreas. Determination made 2 days after operation	0.125	13
2. Same as 1, four days after operation	0.130	10
3. Dog *: Pancreatic ducts ligated 10 weeks before	0.098	73
4. Same as 3, two days later	0.111	98

* The animal went through the usual period of emaciation with recovery and is now well nourished, lively, and behaves like a normal dog, with the exception of having large bulky stools.

9. Milne, L. S., and Peters, H. LeB.: Observations of the Glycolytic Power of the Blood and Tissues in Normal and Diabetic Conditions, J. M. Res. **26**:415 (July) 1912.

10. The method for diastase estimations employed by Milne and Peters is similar to that of Myers and Killian, but not so accurately standardized with respect to quantity of reagents and time of incubation.

determine the effect of a diseased pancreas on the diastatic activity of the blood, estimations of the blood diastase were made in one human case of pancreatic carcinoma found at operation, and in one dog in which the pancreatic ducts had been ligated ten weeks before. The results of these experiments are recorded in Table 7.

It will be seen that no significant changes in the blood diastase were noted in these two instances. However, Myers and Killian,[6] reported a moderate increase in the blood diastase in two cases of pancreatic carcinoma and in two cases of pancreatitis; and it has also already been stated that Block [4] regarded a marked increase in the blood diastase as indicative of pancreatic disease. The diagnostic value of blood diastase determinations in suspected pancreatic disease is therefore at present uncertain.

BLOOD DIASTASE IN NEPHRITIS

Myers and Killian report a decided increase in the blood diastase in eleven of twenty-three cases of nephritis in human beings. Fitz,[11] experimenting on rabbits in which he produced an acute nephritis by

TABLE 8.—*The Diastatic Activity of the Blood in Nephritis*

Case	Diagnosis	Blood Pressure	Phthalein Excretion per Cent.	Blood Urea Nitrogen Mg. per 100 C.c	Blood Sugar	Units of Diastatic Activity
1	Chronic nephritis with hypertension.....	250/145	0	134	0.187	34
(7 days later)	Uremia.....	.....	0	203	0.166	55*
2	Chronic nephritis.....	140/95	Trace	...	0.136	25
3	Subacute nephritis with features of nephrosis (Epstein).	126/90	35	11	0.111	18
4	Chronic nephritis with hypertension.....	180/120	15	33	0.150	15
5	Chronic nephritis with hypertension.....	230/140	Trace	12	0.115	22

* Died 2 days after this determination.

means of uranium nitrate, obtained an increase in the blood diastase (estimated by the Wohlgemuth method) in five out of seven rabbits in which kidney lesions were demonstrated histologically. In the present study the diastatic activity of the blood was estimated in five cases of various types and degrees of nephritis, and the results are recorded in Table 8.

It will be seen that only in one case was a marked increase in the blood diastase found. Two estimations were made in this case at an interval of seven days. At the time of the second examination the patient was in uremic stupor. Although the blood diastase rose from 34 to 56, the blood sugar, at the same time fell from 0.187 to 0.166.

11. Fitz, R.: The Relation Between Amylase Retention and Excretion and Non-Protein Nitrogen Retention in Experimental Uranium Nephritis, Arch. Int. Med. **15**:524 (April) 1915.

Here again the figures speak against any relationship between glycemia and blood diastase. The results, however, seem to indicate that while blood diastase determinations can be of but limited value in the diagnosis of nephritis, they may prove of prognostic value, a steady rise in blood diastase pointing to an unfavorable progress of the disease. Further studies along this line seem desirable.

INCIDENTAL OBSERVATIONS

During the progress of this investigation several problems arose in connection with the technic, which suggested certain experiments. Occasionally it was found necessary to keep a blood specimen from half to one hour before it was possible to begin the diastase determination. In order to ascertain what effect such delay had on the diastatic activity, several experiments were carried out, the results of which are recorded in Table 9. It will be seen from these results that, although

TABLE 9.—*The Stability of the Diastatic Enzyme and its Distribution in the Various Blood Components*

Experiments	Blood Sugar	Units of Diastatic Activity
1. Determination immediately after drawing blood from vein...	0.425	19
2. Same blood as 1, kept 24 hours in refrigerator.....	0.415	11 = 40% loss
3. Immediate determination	0.353	15.4
4. Same as 3, kept 24 hours in refrigerator.....	0.323	15.4 = no loss
5. Immediate determination	0.125	18
6. Same as 5, kept 2 hours in refrigerator.....	0.115	18 = no loss
7. Immediate determination	0.387	17
8. Same as 7, kept 1 hour at room temperature.....	0.387	15 = 12.5% loss
9. Whole oxalated blood.....	0.222	17
10. Same as 9 without oxalate.....	0.222	17
11. Whole oxalated blood.....	0.187	34
12. Plasma from same blood as 11.....	0.166	34
13. Serum from same blood as 11.....	0.166	37
14. Whole oxalated blood.....	0.540	10
15. Serum from same blood as 14.....	0.520	23

a delay of one hour is not likely to result in serious error, it is best to begin the determination within fifteen minutes to half an hour after drawing the blood from the vein.

Another question arose concerning the method of collecting blood, with special reference to the effect which oxalate might have on the enzyme action. Table 9 shows that the oxalate used in this work was without effect on the diastatic action of the blood. Two interesting observations, however, were made concerning the difference in diastatic activity of whole oxalated blood, blood plasma (derived from oxalated blood), and blood serum (derived from clotted blood). Equal quantities (2 c.c.) were used from each of these blood mixtures, and the samples subjected to the same laboratory conditions at the same time.

It will be seen that, although the blood plasma (oxalated) yielded the same results as the whole oxalated blood, yet the blood serum showed a higher diastatic activity than the corresponding whole blood. The

degree of increased activity varied from a very slight gain to more than twice the activity of the corresponding whole blood. This latter observation is interesting, as it suggests the possibility that new diastase may be produced during the process of clotting, perhaps from diastatogenic enzymes which become activated during the process of clotting. It was noted that the degree of increased activity in serum diastase depended on the method of separating the serum, whether the blood was centrifuged immediately on clotting, or whether the serum was permitted to separate slowly. In the latter instance the increase in diastase was greater. Similar observations were noted by Carlson and Luckhardt,[7] although the variations reported by these investigators were less striking. It appears, from these findings, that serum diastase may vary with factors not entirely under control, and is, therefore, less desirable for comparative studies.

SUMMARY AND CONCLUSIONS

1. The diastatic activity of normal human blood was estimated by the Myers and Killian method in thirty-five cases. The figures obtained varied between 2 and 24.

2. The diastatic activity of diabetic blood was estimated, by the same method, in twenty-four cases of human diabetes. The figures obtained were essentially similar to those observed in the normal series, varying between 3 and 26. In one atypical case of diabetes a blood diastase of 32 was observed. The results, furthermore, indicated that a low blood diastase in diabetes was in no way suggestive of an associated syphilitic infection.

3. No significant changes were noted in the diastatic activity of the blood, in serial determinations, on either normal or diabetic subjects, before and after they had taken food.

4. The injection of insulin in two diabetic subjects produced no significant change in the concentration of their blood diastase.

5. There was no correlation noted between the concentration of the blood diastase and the blood sugar concentration in either diabetic or nephritic subjects.

6. The diastatic activity of the blood in two depancreatized dogs was slightly lower than normal during the first two days after the operation.

7. A normal blood diastase was observed in one case of carcinoma of the head of the pancreas. A normal diastatic activity was also obtained in the blood of a dog, in which the pancreatic ducts had been ligated ten weeks before, and in which symptoms of pancreatic insufficiency had been noted.

8. A decided increase in blood diastase was obtained in one case of severe nephritis. The results suggested the possibility that serial determinations in severe nephritis might prove of prognostic value.

9. Estimation of the diastatic activity of the blood, as determined by the method of Myers and Killian, yielded no information of clinical value in the cases of diabetes and in the one case of pancreatic carcinoma studied.

BASAL PULSE RATE AND PULSE PRESSURE CHANGES ACCOMPANYING VARIATIONS IN THE BASAL METABOLIC RATE

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The rôle played by the blood and circulatory system in the gaseous metabolism of the body has constituted the subject of numerous investigations. These fall naturally into two groups. One comprises researches into the oxygen carrying capacity of the hemoglobin and the other group embraces studies on the volume flow of blood. The necessity for a wide range of functional adaptation is apparent when we recall that during severe exercise the amount of oxygen required may be seven or eight times greater than that which suffices under resting conditions.

Barcroft, Haldane and their co-workers demonstrated the reserve power of the hemoglobin by showing that under ordinary conditions oxyhemoglobin gives up approximately only one-third of its oxygen to the tissues but may, when the demand exists, give up much more. Studies on the volume flow of blood, which have been facilitated by the nitrous oxid method of Krogh and Lindhard, indicate that the volume flow of blood may be increased threefold or fourfold during the performance of work. These investigations of two entirely different methods of varying the rate of oxygen transportation by the blood suggest that there exists the mechanism which would provide considerable liability of adaptation. One or possibly both methods are employed whenever larger volumes of oxygen are required under either normal or pathologic conditions. Unfortunately the methods employed in demonstrating the existence of the functions described in the foregoing do not permit of easy clinical application.

Utilizing the respiration calorimeter, it is now possible to measure the oxygen consumption with sufficient accuracy for clinical purposes. Quantitative variations in the amount of work done by the circulatory system in performing its function of transporting oxygen are roughly represented by pulse rate and blood pressure changes, which presumably serve the purpose of altering the volume flow of blood. This report, based on 600 basal metabolic rate estimations, constitutes an endeavor to ascertain the relationship between oxygen consumption and circulatory system activities by the utilization of statistical methods.

The procedure here employed consisted in ascertaining the basal pulse rate simultaneously with determination of the basal metabolic rate. Frequent pulse readings were made throughout the two ten minute periods during which the patient was connected with the respiration

calorimeter. A reading of the blood pressure was made in the resting interval between the breathing tests. This "basal blood pressure," as pointed out by Addis,[1] is lower than the "daytime pressures" and represents more accurately the true status of the person's pressure. The blood pressure readings were recorded, in order to ascertain whether or not the pressure showed any such relationship to metabolic rate as was known to exist between pulse rate and basal metabolic rate.

PULSE RATE

A rapid pulse in a thyrotoxic patient with a high basal metabolic rate is a frequent clinical finding. This association of increased heart rate with elevated metabolic rate has been the subject of repeated comment in the literature. Sturgis and Tompkins [2] reported a study of the correlation of basal metabolism and pulse rate in 154 patients with hyperthyroidism. The opposite finding, a slow pulse rate and low metabolic rate obtaining in hypothyroid persons, is an association commonly observed by workers in clinical calorimetry. That a certain amount of parallelism exists between pulse rate and metabolic rate has been generally recognized. Benedict and Cathcart [3] obtained a very close relationship between the changes in metabolism and pulse rate of a bicycle rider. They report: "It soon became apparent that the pulse rate, even during severe muscular work, very closely followed the amount of energy output, an observation that is fully in accord with the values secured with normal resting subjects which show that the pulse rate and the total metabolism are intimately related." That this association is purposeful, in that the increased number of heart beats serves to hasten the rate of blood flow when more oxygen is required by the tissues, has been demonstrated by many investigators.

PULSE PRESSURE

There are numerous studies reported of the blood pressure in thyrotoxic states since the observations of Gross,[4] in 1900, who found increased systolic pressure in all of his seven patients. The largest

1. Addis, Thomas: Blood Pressure and Pulse Rate Levels, Arch. Int. Med. **29**:539 (April) 1922.

2. Sturgis, C. C., and Tompkins, E. H.: A Study of the Correlation of the Basal Metabolism and Pulse Rate in Patients with Hyperthyroidism, Arch. Int. Med. **26**:467 (Oct.) 1920.

3. Benedict, G., and Cathcart, E. P.: Muscular Work, A Metabolic Study with Special Reference to the Efficiency of the Human Body as a Machine, No. 187, Carnegie Inst., Washington, 1913.

4. Gross, A.: Zur Kenntniss der pathologischen Blutdruckänderungen nach Beobachtungen von Weil, Deutsch. Arch. f. klin. Med. **74**:297, 1902.

series is that of Plummer,[5] who records increased systolic pressure with increased pulse pressure. Many conflicting statements are encountered in the literature on this subject and are due, probably, to two factors: first, the stage of the disease and state of cardiac compensation are not considered and, second, the pressures were taken under varying daytime conditions. One fact seems to be generally established, especially by the later writers, namely, that the pulse pressure is greater than normal, i. e., more than 50 per cent. of the diastolic pressure. This fact is especially stressed by Taussig,[6] who compares the circulatory abnormalities of exophthalmic goiter with those of aortic regurgitation. Harris [7] has recently pointed out the unusual association of rapid pulse and increased pulse pressure and considers it quite characteristic of thyrotoxicosis, since "a frequent pulse in any other condition is usually accompanied by a small pulse pressure." The characteristic sphygmomanometric finding in thyrotoxic states, then, is widened pulse pressure. Accepting this and recalling the normal or low systolic, relatively high diastolic and lessened pulse pressure in hypothyroidism, the idea presents itself that a relationship may exist between pulse pressure and metabolic rate. It was determined, therefore, to limit this study to those blood pressure changes represented by the difference between systolic and diastolic pressure, namely, pulse pressure.

THE DATA

The data constituting the basis of this study were compiled from 600 determinations of the basal metabolic rate. These determinations were made in the departments of clinical calorimetry at St. Luke's Hospital, Stanford University Hospital, the San Francisco Hospital and in private practice. All the work was done with the apparatus described by Benedict as the portable respiration calorimeter. Approximately half of the determinations were made by me, the others by physicians serving their first and second postgraduate years in the before mentioned hospitals.

The subjects furnishing these data were not selected in any way, and the only exclusions were patients who showed pathologic conditions in the cardiovascular-renal system affecting the pulse rate or blood pressure. Table 1 shows the age and sex distribution among the subjects. Ninety-five per cent. were between the age of 25 and 55 years. Exclusion of those above 55 seemed advisable because of the alterations in blood

5. Plummer, H. S.: Blood Pressure and Thyrotoxicosis, Tr. A. Am. Phys. **30**:450, 1915.

6. Taussig, A. E.: Some Blood Pressure Phenomena in Exophthalmic Goiter, Tr. A. Am. Phys. **31**:121, 1916.

7. Harris, I.: The Pulse Pressure in Exophthalmic Goiter, Brit. M. J. **1**: 630 (April 14) 1923.

TABLE 1.—*Age and Sex Distribution*

Basal Metabolic Rate, per Cent.	Female	Male	Years 15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64
70.1 to 80	6	3	0	1	0	0	3	2	3	0	0	0
60.1 to 70	17	5	0	5	4	2	4	3	2	1	0	1
50.1 to 60	23	13	0	3	7	2	9	4	7	4	0	0
40.1 to 50	37	15	0	7	11	7	6	3	11	2	1	3
30.1 to 40	53	20	0	12	15	12	15	2	12	5	0	0
20.1 to 30	49	26	2	9	24	8	10	8	5	6	0	3
10.1 to 20	47	28	4	10	16	10	17	8	5	3	1	1
0.1 to 10	53	28	5	10	16	20	12	8	8	2	0	0
— 9.9 to — 0	44	32	3	9	22	17	8	6	7	4	0	0
—19.9 to —10	46	25	3	7	16	19	10	5	5	5	0	1
—29.9 to —20	11	15	2	6	5	2	6	2	0	2	1	0
—39.9 to —30	1	3	0	1	0	0	0	0	2	1	0	0
Totals.....	387	213	19	80	136	99	100	52	67	35	3	9

pressure so frequently encountered above this age, which are most often due to structural changes in the cardiovascular system. Observations on only three subjects above this age are included, one a hypothyroid man, and two women, both past 60, with typical exophthalmic goiter. A systolic pressure of 160 or over was arbitrarily set as the standard for exclusion from this series, except where a thyrotoxic patient with a high metabolic rate maintained such a pressure with no other evident cause. Electrocardiographic tracings were obtained in approximately two-thirds of the cases.

TABLE 2.—*Averages of Pulse Rate, Pulse Pressure and Their Product in 600 Observations of the Basal Metabolic Rate*

	Basal Metabolic Rate —39.9 to —30%	—29.9 to —20%	—19.9 to —10%	—9.9 to 0.0%	0.1 to 10%	10.1 to 20%	20.1 to 30%	30.1 to 40%	40.1 to 50%	50.1 to 60%	60.1 to 70%	70.1 to 80%
No. of observations	4	26	71	76	81	75	75	73	52	36	22	9
Mean pulse rate.....	59	61	64	71	72	78	81	93	100	104	108	114
Mean pulse pressure	28	32	35	41	43	45	49	51	58	59	69	81
Mean pulse rate × pulse pressure.....	1688	1967	2322	2867	3089	3482	4285	4892	5650	6521	7514	9171

Table 2 shows the data arranged according to the basal metabolic rate into classes, the class interval being made at each 10 per cent. deviation from normal. It will be observed that the mean pulse rates increase as the metabolic rate increases. The same holds true for the pulse pressure. Chart 1 represents the facts recorded in this table. On this graph is also drawn the respective lines of regression for pulse rate and pulse pressure on basal metabolic rate. This line represents the ideal straight line which would be obtained from the averages of an enormous number of observations. In order to obtain this line it was necessary to calculate the coefficient of correlation between pulse rate and basal metabolic and between pulse pressure and metabolic rate. These figures were, respectively, 0.74 and 0.62. This coefficient is based on unity as

representing perfect correlation, and the figures obtained here would be interpreted as indicating that there is a marked correlation between both pulse rate and pulse pressure and metabolic rate, the relationship being somewhat closer for pulse rate than for pulse pressure.

Table 2 gives only means for large groups and tells us nothing about individual cases, how closely the individual readings approximated the mean, or what the variation was within a group. An idea of this degree of variation is obtained from Table 3, which records the coefficients of variation for pulse rate and pulse pressure. It will be observed that pulse pressure is slightly more variable than pulse rate, that with seventy or more observations these values tend to be rather constant

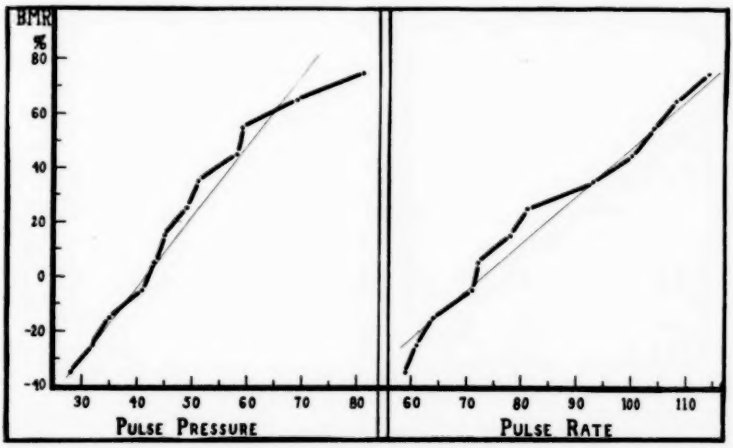

Chart 1.—The dots, connected by the heavy line, represent the mean values for pulse pressure and pulse rate as they are affected by the basal metabolic rate. The data are in Table 2. The light, straight line is the line of regression for pulse pressure and pulse rate, respectively, on basal metabolic rate and is the best fitting straight line which can be drawn through the dots. It is the ideal "line of means."

and that with the higher metabolic rates the variability tends to increase.

Fluctuation in pulse rate and pulse pressure under the conditions of these observations is a significant fact and suggests further study. Several questions arise at once. Do all persons show a rise in both pulse rate and pulse pressure with elevated basal metabolic rate? Is the rise in each proportionate to the other and to the increase in rate? The first question may be answered by saying that no person was observed who did not show an increase in either pulse rate or pulse pressure with increased oxygen consumption. The answer to the second question would read that most individuals responded to increased oxygen con-

sumption with proportionate rises in pulse rate and pulse pressure, but some showed disproportionate rises in one or the other. For example, of two patients with basal metabolic rates of plus 34.6 per cent. and 35 per cent., the former had a pulse rate of 102 while the latter's pulse rate was only 68, but their respective pulse pressures were 42 and 70 mm. of mercury. Or again of two patients with metabolic rates of plus 67 per cent. and 69 per cent., one had a pulse rate of 112 and pulse pressure of 48, while the pulse rate of the other was 85 with a pulse pressure of 94. These are extreme cases selected to illustrate the point under discussion, but this same phenomenon has been observed and commented upon by others.[8] There are many such cases in this series, in which the pulse rate was below its mean and the pulse pressure above its mean for that metabolic rate class, or vice versa. In other words, many individual cases were observed in which the deviations of pulse rate and pulse pressure from their respective means were in opposite directions.

TABLE 3.—*Coefficients of Variation for Pulse Rate, Pulse Pressure and Their Product*

	Basal Metabolic Rate										
	—39.9 to —30%	—29.9 to —20%	—19.9 to —10%	—9.9 to 0.0%	0.1 to 10%	10.1 to 20%	20.1 to 30%	30.1 to 40%	40.1 to 50%	50.1 to 60%	60.1 to 70%	70.1 to 80%
No. of observations	4	26	71	76	81	75	75	73	52	36	22	9
Pulse rate	—	15.32	15.06	13.33	13.33	13.33	17.65	16.66	13.00	17.01	18.37	—
Pulse pressure	—	31.25	21.85	20.73	17.74	20.88	24.08	29.21	22.88	30.67	22.53	—
Pulse rate × pulse pressure	—	33.72	24.98	24.78	21.94	23.49	28.93	33.32	26.53	40.41	25.95	—

These pulse rates and pulse pressure deviations in opposite directions from their means were of all degrees, but in 3.5 per cent. of all observations their magnitude was 20 per cent. or more from their respective means. This produced to a large extent the variability which is shown in Table 3. It is interesting to note that the coefficients here obtained correspond very closely with those obtained by Addis[9] in 300 normal persons.

It is a matter of interest to obtain the measure of the relationship between pulse rate and pulse pressure in this series. This is given by the coefficient of correlation between these two variables. It is noted from Table 4 that this is 0.489 and is positive, indicating that as pulse rate increases there is a decided tendency toward an elevation also in pulse pressure. This is the relationship as it exists in this series in which the basal metabolic rate is not constant but increases by 10 per cent. at each

8. Sturgis, C. C.: Observations on One Hundred and Ninety-Two Consecutive Days of the Basal Metabolism, Food Intake, Pulse Rate, and Body Weight in a Patient with Exophthalmic Goiter, Arch. Int. Med. **32**:50 (July) 1923.

9. Addis, Thomas: Blood Pressure and Pulse Rate Reactions, Arch. Int. Med. **30**:240 (Aug.) 1922.

class interval. It is possible to obtain a *partial* coefficient of correlation in which the factor of basal metabolic rate is held constant and its influence on pulse rate and pulse pressure eliminated. This was done and the very low partial coefficient of correlation of 0.045 obtained, which indicates that there is practically no correlation between pulse rate and pulse pressure or, in other words, where there is a tendency for them both to increase in the same direction, this effect is neutralized by those instances where they vary in opposite directions. If they all, or a majority, showed this tendency to vary in opposite directions, then the coefficient would be preceded by a negative sign, instead it has a positive sign but is almost zero.

RECAPITULATION

We may recapitulate at this point by stating that both pulse rate and pulse pressure vary directly with the oxygen consumption, but the amount of variation in each is not always proportional but nevertheless there seems to be a compensating mechanism at work; for example, a disproportionate increase in pulse rate is compensated for by a relative decrease in pulse pressure or vice versa. These observations suggest that a combination of pulse rate and pulse pressure would give a more correct estimate of the circulatory system's response to varying volumes of oxygen consumed than is afforded by either one alone. An investigation of this point was undertaken and a conclusion reached after study and trial of various methods. The two most feasible methods will be briefly discussed.

PULSE RATE AND PULSE PRESSURE COMBINED

The first method which suggests itself is to combine pulse rate and pulse pressure by multiplying them together. There is ample justification for this procedure, since the product of pulse rate by pulse pressure has been used by many investigators as a measure of the volume flow of blood and the study of this relationship has given rise to an extensive literature.

Erlanger and Hooker [10] assumed that the pulse pressure represented the systolic output of the heart and, in 1904, utilized "the product of the pulse pressure by the pulse rate as an index to the relative velocity of blood flow." The "amplitude-frequency product" was also used by von Recklinghausen [11] in 1906, though it was probably first suggested by Hürthle in 1901. The pulse rate pulse pressure product has recently

10. Erlanger, J., and Hooker, D. R.: An Experimental Study of Blood Pressure and of Pulse Pressure in Man, Johns Hopkins Hosp. Rep. **12**:145, 1904.

11. Von Recklinghausen, H.: Was vir durch die Pulsdruckkreis & durch die Pulsdruckamplitude uber den grossen Kreislauf erfahren, Arch. f. exper. Path. u. Pharmokol. **56**:1, 1906-1907.

been used by Addis,[1] who shows that in normal subjects the product increases under conditions, such as exercise, which call for increased volume flow of blood.

More pertinent to the subject of this communication, however, are the observations of Murlin and Greer,[12] who report their work on dogs as well as on several men. They conclude: "The results show a fairly close correlation in the same individual between the heart output expressed as the product of the pulse pressure and the heart rate on the one hand and the absorption of oxygen and the elinmination of carbon dioxid on the other."

In this study the product in each individual observation was obtained and the means for each group calculated. These are recorded in Table 2. It was observed that the plotted line of these mean products was smoother than the mean lines for either pulse rate or pulse pressure. This was naturally to be expected since the small variations in either pulse pressure or pulse rate would tend to be equalized by variations in the opposite direction in the other factor. This was an advantage, and in individual instances accomplished the desired purpose of combining pulse rate and pulse pressure so as to get an expression which took cognizance of both, regardless of any variability in either. However, it also gave relative importance to each according to its numerical value, which was not desirable in all cases since we know that there is closer correlation between pulse rate and basal metabolic rate than there is between pulse pressure and metabolic rate.

There are two fundamental objections to the use of the product of pulse rate by pulse pressure. One is that it is more variable than either one of its components, as is demonstrated by the coefficient of variation shown in Table 3. This naturally results from the multiplication of small differences, which might be due to error, by large figures, 100 or more in some instances. Another objection to the product is that its use converts a linear relationship into one which is definitely non-linear. The line plotted from the mean products in Table 2 is definitely a curved line, whereas the lines of means for pulse rate and pulse pressure are apparently straight lines. This fact is also demonstrable statistically by employment of methods advanced by Blakeman,[13] the data for calculating which may be obtained from the statistical values obtained in this study and recorded in Table 4. By this test pulse rate is perfectly linear while pulse pressure shows only a slight tendency to

12. Murlin, J. R., and Greer, J. R.: The Heart Action in Relation to the Respiratory Metabolism, Proc. Am. Physiol. Soc., **18**, 1910; Am. J. Physiol. **27**: 1910-1911.

13. Blakeman, J.: On Tests for Linearity of Regression in Frequency Distributions, Biometrika **4**:332, 1905.

deviate from linearity. The nonlinearity of the product of pulse rate and pulse pressure is confirmed by this test.

There is one further objection to the utilization of the product as a means of combining the two variables, pulse rate and pulse pressure. It has come to represent a rough measure of the volume flow of blood, the pulse rate indicating the rate of flow and the pulse pressure the systolic output of the heart. There is no objection to assuming that the rate of heart beat influences rate of blood flow, but many careful investigators claim that pulse pressure is not an indication of systolic cardiac output and Yandell Henderson, who is supported by Haldane, maintains that the systolic cardiac output is a constant and is so maintained under a variety of conditions. On the other hand, Means and Newburgh [14] have shown that an increase in the systolic output may be a factor in increasing the volume flow of blood. These investigators remark in

TABLE 4.—*Statistical Values and Relationships*

Symbol	M	σ (sigma)	ρ (rho)		η (eta)	ζ (zeta)
	Designation					
		Standard Deviation	Coefficient of Correlation: With Basal Metabolic Rate	Coefficient of Correlation: With Pulse Pressure	Correlation Ratio With Basal Metabolic Rate	
	Mean					η^2-ρ^2
Basal metabolic rate (0)	16.9	25.2	.....	.....	.....	.....
Pulse rate (1)	82.4	19.3	0.745 ± 0.013	$0.489 \quad 0.021$	0.745	0
Pulse pressure (2)	47.9	15.5	0.625 ± 0.015	.....	0.633	0.01006 ± 0.0055
Pulse rate × pulse pressure	4030	2060	0.759 ± 0.012	.....	0.804	0.07063 ± 0.0145

discussing one of their subjects: "Up to 700 kilogram-meters of work per minute the increase in blood flow was met chiefly by an increase in the volume per beat; beyond that point by an increase in the pulse rate." It must be remembered that they were measuring the systolic output of the heart by an objective method, which lends support to the assumption that changes in pulse pressure indicate variations in the systolic output of the heart.

That variations in pulse pressure as well as pulse rate occur, and that there seems to be some regularity in their occurrence is quite apparent from this and other studies. But, since these variations, though they appear to be purposeful, are not thoroughly understood, it was considered advisable to treat them in an abstract manner simply as variables and combine them in the manner that seemed most expedient irrespective of any theory or interpretation as to their significance.

It has been mentioned that the coefficient of correlation between pulse rate and basal metabolic rate was 0.74, and the coefficient for pulse pres-

14. Means, J. H., and Newburgh, L. H.: Studies of the Blood Flow by the Method of Krogh and Lindhard, Tr. A. Am. Phys. **30**:51, 1915.

sure and metabolic rate was 0.62. It is possible to calculate a multiple coefficient of correlation which utilizes the relationships existing between pulse rate, pulse pressure and basal metabolic rate and also between pulse rate and pulse pressure themselves. This was done and a multiple coefficient of correlation of 0.80 was obtained. This is appreciably higher than the coefficient of correlation for pulse rate or pulse pressure alone, and may be taken as an indication of the advantage to be gained by combining the values for these two functions.

In a previous communication [15] on this subject, a doubt was expressed as to whether the relationship existing between basal metabolic rate and pulse rate combined with pulse pressure was truly linear or curvilinear. From further study with twice the number of observations it now becomes apparent that a straight line most nearly represents the facts. Such a straight line may be obtained by utilization of the relationships which are revealed by a statistical analysis of these data. The values are all contained in Table 4 and may be substituted in the following equation:

$$1.\ \chi_0 = \left[\beta_{01\cdot 2}\,\frac{\sigma_0}{\sigma_1}\right]\chi_1 + \left[\beta_{02\cdot 1}\,\frac{\sigma_0}{\sigma_2}\right]\chi_2 + \left[M_0 - \beta_{01\cdot 2}\,\frac{\sigma_0}{\sigma_1}\,M_1 - \beta_{02\cdot 1}\,\frac{\sigma_0}{\sigma_2}\,M_2\right]$$

In which $\beta_{01\cdot 2} = \frac{r_{01} - r_{02}\,r_{12}}{1 - r^2_{12}}$ and $\beta_{02\cdot 1} = \frac{r_{02} - r_{01}\,r_{12}}{1 - r^2_{12}}$

Substituting the values and reducing to its simplest form this equation becomes:

$$2.\ \text{B.M.R.} = 0.75\ (\text{P.R.} + 0.74\ \text{P.P.}) - 72.$$

From such an equation a straight line may be plotted which represents the relationship between basal metabolic rate on the one hand and pulse rate and pulse pressure on the other. This line has been drawn in Chart 2 and on it have been placed the 600 observations of pulse rate and pulse, pressure from whose values and relationships the line is derived.

Careful inspection of Chart 2 suggests the possibility that a curved line could be drawn through the dots which would more nearly pass through the areas of their greatest concentration. In order to ascertain this point with certainty the means of the columns 70-80, 80-90, etc., in Chart 2 were obtained and are placed as crosses on this graph with the best fitting curve drawn through them. It becomes evident from inspection of these lines that in the metabolic rate classes above plus 20 per cent. there is a tendency for the means to deviate from the straight line. This same tendency toward a wider divergence in the readings

15. Read, J. M.: Correlation of Basal Metabolic Rate with Pulse Rate and Pulse Pressure, J. A. M. A. **78**:1887 (June 17) 1922.

obtained from patients with the higher basal metabolic rates is noted in the coefficients of variation (Table 3), which increase in magnitude with the higher metabolic rates. This point is shown graphically by the increased scatter of the dots in the upper part of Chart 2.

In view of the marked linearity in the relationship between pulse rate and pressure and basal metabolic rate, with rates up to plus 20 per cent., it would seem pertinent to inquire for a cause of the deviation from linearity noted with the higher metabolic rates. There is especially noted a tendency, beginning at about plus 55 per cent., for the combined

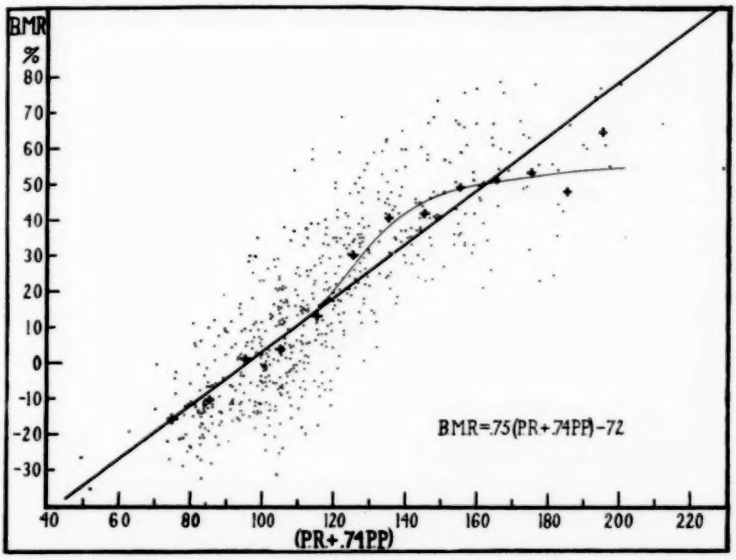

Chart 2.—The heavy straight line is the line of regression for combined pulse rate and pulse pressure on basal metabolic rate and is represented by the equation: B.M.R. = 0.75 (P.R. + 0.74 P.P.) — 72. The 600 dots represent the individual observations from whose values and relationships the line was derived. The crosses are the means of the columns, and the curved line passing through them in the upper portion illustrates the extent of the tendency for pulse rate and pulse pressure to deviate from linearity when the metabolic rate is above plus 20 per cent. It is also to be observed that above this point there is greater variability, as evidenced by the increased scatter of the dots.

pulse rate and pulse pressure figures to increase out of all proportion to increase in the basal metabolic rate. The patients with these higher metabolic rates were all suffering from so-called hyperthyroidism and in this fact probably lies the cause of the observed phenomenon. Cardiac decompensation, however, is not a factor, as no thyrotoxic patients with

myocardial failure or auricular fibrillation are included in this series. It seems more probable that factors inherent to the disease itself are operating to cause the changes noted.

The apparently ineffectual increase of the work of the circulatory system when the metabolic rate becomes high brings to mind a recent report by Plummer and Boothby,[16] in which they showed that patients with exophthalmic goiter were very inefficient as machines; such patients expending about twice as much energy as normal persons doing the same work. All these facts taken together would seem to indicate that exophthalmic goiter is not simply hyperthyroidism. If it were, the same linear relationship should hold for the higher metabolic rates as holds for rates obtained from normal and hypothyroid subjects. When thyrotoxic patients are the subjects there may be, and probably are, other factors which are operating to increase the pulse rate, namely, toxemia and, perhaps, increased sympathetic instability. These factors also affect blood pressure but in a manner more difficult to appraise. Inspection of Table 3 and Chart 2 certainly reveals wider variability in pulse rate and pulse pressure with increasing metabolic rate.

PRACTICAL APPLICATIONS

It has been suggested [17] that the resting pulse rate be utilized as an index of the basal metabolic rate. Means and Aub [18] found that in about 60 per cent. of cases studied by them "there is a close parallelism between pulse and metabolism, and in the remainder a certain amount of parallelism." They further state: "The pulse rate, however, gives comparative results only, for between metabolism elevation of different persons there seems to be but little relationship. The pulse rate, therefore, is not an index of the absolute degree of intoxication in the patient seen at different times." These statements concerning pulse rate are equally true of pulse pressure. Hence it follows that if an index of basal metabolic rate is sought, the value of such index is enhanced by ultilization of pulse pressure as well as pulse rate. First the best means of combining these values must be decided. Although a curved line seems to fit best the data here reported, still there is the difficulty that a formula representing such a line becomes too complicated for practical use. This same result could be obtained by utilizing Chart 2 directly, and noting the relation of the dot obtained to the curved line of the means. Because of its simpler application, however, the linear formula is suggested as

16. Plummer, H. S., and Boothby, W. M.: The Cost of Work in Exophthalmic Goiter, Am. J. Physiol. **63**:406-407 (Feb.) 1923.

17. The Pulse Rate in Relation to Metabolism and Diagnosis, Editorial, J. A. M. A. **76**:181 (Jan. 15) 1921.

18. Means, J. H., and Aub, J. C.: The Basal Metabolism in Exophthalmic Goiter, Arch. Int. Med. **24**:645 (Dec.) 1919.

the most useful for clinical work if a prediction formula is desired. So by substituting pulse rate and pulse pressure readings in the foregoing equation (*2*) an estimation of the basal metabolic rate may thereby be obtained. Inspection of Chart *2* shows that only a little over half of the observations would fall within 10 per cent. above or below the correct value. This is no objection, however, to utilization of pulse rate and pulse pressure as a rough guide in following the course of a hypothyroid or thyrotoxic patient who is under treatment. The reliability of the figures becomes greatly enhanced by obtaining them at an examination, simultaneously with an estimation of the basal metabolic rate, and thus establishing the relationship which exists *in that person* between pulse rate, pulse pressure and basal metabolic rate. When the pulse rate and blood pressure are obtained under the same basal conditions necessary for satisfactorily determining a basal metabolic rate these values would seem to be sufficiently reliable to warrant their use where it is not practical to obtain frequent determinations of the basal metabolic rate.

CONCLUSIONS

1. Pulse rate varies directly with the basal metabolic rate, the coefficient of correlation between pulse rate and basal metabolic rate being 0.74.

2. Pulse pressure also varies directly with the basal metabolic rate, the coefficient of correlation in this case being 0.62.

3. Since different persons vary in the amount of change in either pulse rate or pulse pressure, a more reliable measure of the circulatory system's response to variations in oxygen consumption is a combination of pulse rate and pulse pressure in the manner suggested below.

4. Pulse rate and pulse pressure changes may be utilized as a rough guide to changes in a patient's basal metabolic rate, particularly becoming more reliable if the relationship to metabolic rate is once established by obtaining all the basal figures simultaneously. By substituting the basal pulse rate and pulse pressure readings obtained in any given case in the following formula, an estimate of the basal metabolic rate may be obtained:

$$\text{B. M .R.} = 0.75\ (\text{P. R.} + 0.74\ \text{P. P.}) - 72.$$

This will give the basal metabolic rate withing 10 per cent. of its correct value in slightly over half of the cases.

THE ALLEGED RÔLE OF LACTIC ACID IN ARTHRITIS AND RHEUMATOID CONDITIONS *

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In 1858 Richardson [1] published the results of extensive experiments on dogs in which the injection of large quantities of lactic acid, intraperitoneally, was followed by severe joint involvement. The condition of the joints was similar to that seen in acute arthritis, and Richardson suggested that the arthritic syndrome was due to an accumulation of lactic acid in the body. This theory found further support in 1877, when Foster [2] reported that the administration of lactic acid by mouth to two diabetic patients resulted in painful and swollen joints. The pain and swelling persisted as long as the lactic acid administration was continued and disappeared promptly after the acid was discontinued. These early experiments were apparently never repeated or extended but they have exerted some influence in the formation of hypotheses regarding the disease. Within very recent years, for example, the theory that arthritis and muscular rheumatism are characterized by an excessive accumulation of lactic acid in the body has been revived, chiefly on clinical grounds, by Wilde, and has found expression in his book on arthritis.[3]

Moreover, in view of the known relation of lactic acid to muscular contraction and rigor mortis; and in view of the peculiar dysfunction characteristic of rheumatic myositis and the beneficial influence on it of passive motion, massage and active motion, there has been ample basis on which to erect an hypothesis correlating the disease process with a disturbed production or removal of lactic acid. It is conceivable that if such a disturbance exists there would be a reasonable probability of detecting it by a determination of the lactic acid in the blood and other body fluids.

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* From the Laboratory of Clinical Chemistry, Presbyterian Hospital. The work here reported is part of a study on chronic arthritis in collaboration with Robert B. Osgood, M.D., of Boston. The expenses of the investigation were defrayed by contributions from several sources, including a number of patients.

1. Richardson, B. W.: The Cause of the Coagulation of the Blood. The Astley Cooper Prize Essay for 1856, London, John Churchill, 1858, Appendix III, p. 371.

2. Foster, B.: Clinical Medicine, London, J. and A. Churchill, 1874, p. 143.

3. Wilde, P.: Physiology of Gout, Rheumatism and Arthritis, New York, William Wood & Co., 1922.

According to the present conception of the physiologic significance of lactic acid, excessive production of this acid or faulty removal of it from the body would indicate marked abnormal oxidative function, either in respect to intermediary glucose metabolism or to products of the energy transformation in muscular and perhaps glandular activity. In order to ascertain whether there occurs in arthritis such deviation from normal physiology as is implied by the lactic acid theory, it has seemed desirable to test this theory by chemical determination of the quantities of lactic acid present in the body fluids of arthritic patients and normal persons. Methods for the determination of lactic acid are at present available whereby the lactic acid present in small amounts of biologic material can be quantitatively evaluated with a fair degree of accuracy.

EXPERIMENTAL

Persons suffering from acute or chronic arthritis were used as subjects in this investigation. Both proliferative and degenerative types with widely varying degrees of involvement and disability were represented in this group. The normal subjects were drawn from convalescent surgical cases and various hospital workers who apparently were in good health. Venous blood samples were drawn from the cubital vein usually in the post-absorptive period and always after a period when excessive muscular activity had been avoided. The urine analyses were made on twenty-four hour samples, and on samples voided before and after exposure to external heat. Sweat samples were collected in a rubber bag surrounding the forearm during a general sweat induced by an electric "baker." The skin of the arm and hand was cleaned with water, alcohol and ether before inserting the limb into the bag. One sample of nonseptic joint fluid was obtained by aspiration from an arthritic knee joint.

For the determination of lactic acid Clausen's method has been used.[4] This method has proved fairly satisfactory in our hands. Using a metal alloy bath and sulphuric acid to decompose the lactic acid to acetaldehyd it was possible to recover an average of 94 per cent. of the lactic acid contained in lithium lactate solutions and about the same proportion of lactic acid added to blood. The use of traps to avoid mechanical passage of droplets from the reaction tube to the bisulphite solution and the maintenance of the latter at a p_H of 3.0 to 5.5 are details that Clausen has suggested as contributory towards uniform results.[5]

Clausen's directions have been followed in the treatment and analysis of blood and urine. Sweat samples have been prepared for analysis by precipitating the small amount of protein present with

4. Clausen, S.W.: J. Biol. Chem. **52**:263 (May) 1922.
5. Personal Communication.

tungstic acid in the manner customary for blood proteins. In none of the sweat samples that we tested have reducing sugars been found and, consequently, the preliminary removal of glucose, necessary with blood filtrates, was dispensed with. Nearly identical results for lactic acid were obtained from tungstic acid filtrates prepared in this way and from ether extracts of sweat.

RESULTS AND DISCUSSION

As will be seen from Table 1, the content of lactic acid in the venous blood of arthritic patients is quantitatively similar to the blood content of lactic acid of normal persons. These figures are of the same magni-

TABLE 1.—*Lactic Acid Content of Whole Blood*

Name	Diagnosis †	Lactic Acid, Mg. per 100 C.c.	Remarks
*Sh-e	Proliferative arthritis	23	
*L-s	Arthritis	21	After 10 gr. acetylsalicylic acid
*F-ll	Proliferative arthritis	20	After 10 units insulin
*F-ll	Proliferative arthritis	16	After 10 units insulin
*A-x-r	Proliferative arthritis	26	
S-t-n	Proliferative arthritis	26	
*Sch-d	Degenerative arthritis	19	
*D-n-d	Degenerative arthritis ?	17	
*D-n-d	Degenerative arthritis ?	18	After "bake"
P-t-r	Subacute rheumatic fever	11	
V-s	Proliferative arthritis	23	
V-s	Proliferative arthritis	22	
*C-y-e	Proliferative arthritis ?	25	
D-l-p	Psoriasis and diabetes	30	
K-g-e	Arthritis	19	
C-l	Normal	20	
C-ll	Proliferative arthritis	21	
C-ll	Proliferative arthritis	19	
Cr-t-r	Normal	16	
C-r-ll	Malignant tumor of liver	16	
C-l	Normal	20	

† An attempt was made to classify the arthritic patients studied, according to the classification of Nichols and Richardson, into the proliferative and degenerative types, but this was not always clinically possible. Practically all subjects presented some symptoms of rheumatic myositis. This was conspicuous in the cases starred.

tude as reported by other investigators. Clausen finds a lactic acid range of from 15 to 32 mg. per hundred cubic centimeters in convalescent children at rest in bed. Barr, Himwich and Green,[6] using Clausen's method, found from 14 to 25 mg. lactic acid in arterial blood of resting persons. After three minutes exercise the lactic acid in the blood of these same persons rose to levels from three to five times higher than the resting level. These experiments, just cited, demonstrate clearly that excessive production of lactic acid, in muscle tissue at least, is promptly reflected in the blood, and it is difficult to escape the conclusion that if there is excessive production of lactic acid in the course of the arthritic or rheumatic process it would be revealed in abnormally high

6. Barr, D. P.; Himwich, H. E., and Green, R. P.: J. Biol. Chem. **55**:495, 1923.

values in the circulating blood. It is of interest, perhaps, to note that the lowest blood lactic acid recorded in Table 1, was from a case of non-febrile acute rheumatism where muscular movement probably was at a minimum because of the extreme pain involved. A value of 28 mg. of lactic acid per hundred cubic centimeters of fluid was obtained in the one sample of nonseptic joint fluid studied.

In Table 2 are given a few determinations of lactic acid in urine. We have been unable to obtain residues from ether extracts of urine that did not char considerably in the reaction tube with sulphuric acid, and we are unwilling to place much reliance on the figures as representing

TABLE 2.—*Urinary Excretions of Lactic Acid and Organic Acids in Arthritis*

Name	Total Volumes, C.c.	Hourly Volume, C.c.	p_H	Lactic Acid Mg. per 100 C.c.	Lactic Acid Mg. per Hour	Organic Acids C.c. of 0.1 N per Hour	Remarks
Sh-e.....	62	53.1	6.8	18	9	6.4	Before "bake"
Sh-e.....	58	69.6	6.8	20	14	7.7	After "bake"
Sh-e.....	1,250	52.1	6.4	15	8	18.0	24 hour sample
Sh-e.....	1,180	49.2	6.2	26	13	15.3	24 hour sample day of "bake"
Sh-e.....	1,290	53.8	6.2	11	6	19.3	24 hour sample
L-s.....	1,805	75.2	6.8	8	6	21.8	24 hour sample
L-s.....	1,058	44.1	6.0	13	6	17.9	24 hour sample day of "bake"
L-s.....	765	31.9	5.8	18	6	21.0	24 hour sample
S-ek.....	35	30.0	5.6	14	4	13.5	Before "bake"
S-ek.....	24	22.2	5.6	15	3	10.9	After "bake"
H-y.....	134	89.3	5.6	23	21	25.5	Before "bake"
H-y.....	102	72.0	7.2	21	15	30.2	After "bake"
H-y.....	61	52.2	5.8	31	16	31.8	Before "bake"
H-y.....	110	62.8	6.6	22	14	35.8	After "bake"
B-s.....	1,873	78.0	...	..	..	12.6	24 hour sample
B-s.....	1,840	76.7	...	..	..	14.4	24 hour sample
B-s.....	2,305	96.0	...	.	.	18.3	24 hour sample
C-t-n.....	1,320	55.0	...	..	..	11.8	24 hour sample
C-t-n.....	1,625	67.7	...	..	..	14.2	24 hour sample
A-r-n.....	1,210	50.4	...	..	..	11.5	24 hour sample
A-r-n.....	1,565	65.2	...	..	..	11.9	24 hour sample
S-p-d.....	1,150	47.9	5.4	..	..	15.1	24 hour sample
S-p-d.....	1,410	58.8	6.2	..	..	17.1	24 hour sample
S-p-d.....	1,270	52.9	6.0	..	..	14.0	24 hour sample
S-p-d.....	975	40.6	6.4	..	..	16.4	24 hour sample

lactic acid alone. However, there is no indication of excessive amounts of bisulphite binding compounds in these ether extracts. The excretion of large amounts of lactic acid in urine would be revealed in the titration for organic acids as carried out by the procedure of Van Slyke and Palmer.[7] These authors have shown that lactic acid is titrated to an extent of 93 per cent. in their method. Many titrations for organic acids have been made in arthritic urines without finding abnormally high values. Some of these results are included in Table 2.

Particular attention has been paid to the sweat since Wilde ascribes the value of sweating measures in arthritis largely to the elimination of lactic acid in the sweat. As will be seen from Tables 3 and 4 considerable quantities of lactic acid were found in sweat, but the sweat of arthritic patients contained no more than the sweat of normal persons.

7. Van Slyke, D. D., and Palmer, W. W.: J. Biol. Chem. **41**:567 (April) 1920.

The quantity of lactic acid present in the sweat of both groups varies over a wide range and the concentration in any sample seems dependent on the dilution of the sweat. The water content of sweat increased

TABLE 3.—*Lactic Acid Content of Arthritic and Normal Sweat*

Name		Diagnosis	p_H	Lactic Acid Mg. per 100 C.c.	Total Solids, per Cent.	Ash, per Cent.
M-n	(a)*	Normal	6.6	287	1.32	0.88
	(b)*		7.8	120	0.85	0.79
C-l	(a)	Normal	7.4	186	1.13	0.81
	(b)		7.6	136	1.01	0.83
P-t-n	(a)	Normal	4.8	350	1.19	0.39
	(b)		6.4	171	0.73	0.61
P-e	(a)	Arthritis	6.2	234	1.17	0.62
	(b)		6.5	145	0.90	0.66
M-l-n	(a)	Arthritis	6.0	458	1.49	0.61
	(b)		6.0	...	1.07	....
Seh-d	(a)	Arthritis	7.2	206	0.98	0.71
	(b)		7.4	148	0.74	0.64
Ar-t		Normal	6.6	166	....	....
A-n		Normal	...	248	....	....
M-ll		Normal	...	424	....	....
H-n-n		Arthritis	6.4	196	....	....
G-s		Arthritis	6.8	139	....	....
M-t-f		Arthritis	6.8	104	....	....
W-e		Nephritis	5.8	212	....	....
R ph		Psoriasis	5.4	130	....	....
F-s		Arthritis	5.8	200	....	....
S-ek		Arthritis	6.0	184	....	....
Sh-e		Arthritis	6.9	164	....	....
L-s		Arthritis	6.4	226	....	....
K-l-r		Arthritis	5.2	229	....	....
D-n-d		Arthritis	6.8	207	....	....
F-ty		Arthritis	6.1	208	....	....

* (a) and (b) refer respectively to sweat samples taken early and late in the course of the electric "bake."

TABLE 4.—*Lactic Acid and Total Organic Acid Content of Arthritic Sweat*

Name	Diagnosis	p_H	Lactic Acid Mg. per 100 C.c.	Lactic Acid C.c. of 0.1 N per 100 C.c.	Organic Acids C.c. of 0.1 N per 100 C.c.	Per Cent. Organic Acids as Lactic Acid
S-ek	Arthritis	6.0	184	20	35	57
D-n-d	Arthritis	6.8	207	23	34	78
D-n-d	Arthritis	6.5	230	26	40	65
R-t-r	Arthritis and psoriasis	6.8	149	17	27	63
R-t-r	Arthritis and psoriasis	5.6	98	11	23	48
R-t-r	Arthritis and psoriasis	6.8	186	21	31	68
R-t-r	Arthritis and psoriasis	7.0	159	18	31	58
R-t-r	Arthritis and psoriasis	6.6	250	28	50	56
R-t-r	Arthritis and psoriasis	7.0	151	17	52	33
R-t-r	Arthritis and psoriasis	7.0	146	16	34	47
Seh-d	Arthritis	7.0	126	14	27	52
Seh-d	Arthritis	6.8	136	15	21	71
Seh-d	Arthritis	...	113	13	52	25
Seh-d	Arthritis	...	90	10	39	24
M-t-f	Arthritis	6.6	131	15	25	60

appreciably as the individual continued to sweat. The diminution of solids seems to be largely a loss of the organic solids. Little change in the ash concentration was found, though occasionally an increase in late samples. This is in keeping with the observation of Adolph [8] that

8. Adolph, E. F.: Am. J. Physiol. **66**:445 (Nov.) 1923.

the chlorid content of sweat increases during sweating, reaching a plateau after from twenty to thirty minutes of sweating. The figures for the ash content of sweat are considerably higher than the recently published results of Talbert.[9] The range of lactic acid figures found for arthritic patients and normal persons, and given in the various tables, are summarized as follows:

Arthritis patients: Blood; from 11 to 30 mg. per hundred cubic centimeters. Urine; from 3 to 21 mg. per hour; from 8 to 31 mg. per hundred cubic centimeters. Sweat; from 90 to 458 mg. per hundred cubic centimeters.

Normal persons: Blood; from 14 to 25 mg. per hundred cubic centimeters, from 15 to 32 mg. per hundred cubic centimeters. Barr, Himwich and Green;[3] Clausen.[4] Urine; from 5 to 13 mg. per hundred cubic centimeters. Sweat; from 120 to 424 mg. per hundred cubic centimeters.

In a number of cases when large quantities of sweat were available, an approximation of the total organic acid content was obtained by titration. Van Slyke and Palmer's procedure for organic acids in urine was used,[7] and the titration carried out on 10 c.c. samples of sweat. The results, given in Table 4, indicate that lactic acid makes up about one half of the total organic acids contained in sweat.

As early as 1852, Favre [10] isolated the zinc salt of lactic acid and identified lactic acid as a sweat constituent. Ryffel,[11] as a result of one analysis by his own method, also reported its presence in sweat collected during a Turkish bath. Clausen has emphasized the fact that his method is not specific for lactic acid and in order to satisfy ourselves that we were actually dealing with lactic acid in appreciable amounts, large quantities of acidified sweat were extracted with ether and lactic acid was isolated from it as zinc lactate. By analysis of this zinc salt, the lactic acid in sweat was identified as *d*-lactic acid. The yield was about 50 per cent. of the amount indicated by Clausen's method as being present.

0.1062 gm. substance after drying to constant weight at 105 C. weighed 0.0925 gm.
0.0402 gm. anhydrous substance gave 0.0137 gm. zinc oxid (ZnO).
0.0244 gm. anhydrous substance gave 0.0083 gm. zinc oxid (ZnO).
0.1936 gm. in 14.23 gm. water in 20 cm. tube: $\alpha = -0.3$ degrees.
Calc. for Zn $(C_3H_5O_3)_2$ $2H_2O$, water = 12.88 per cent.
Found water=12.90 per cent.
Calc. for Zn $(C_3H_5O_3)_2$ zinc oxid = 33.43 per cent.
Found zinc oxid=34.08, 34.02 per cent.
(α) $D^{15°} = -11.1$ (c = 1.25 per cent.) (Beilstein, **3**:263, 1921).
Found (α) $D^{20°} = -11.0$ (c = 1.36 per cent.).

9. Talbert, G. A.: Am. J. Physiol. **63**:350 (Jan.) 1923.
10. Favre, M. P. A.: Compt. rend. Acad. d. sc. **35**:721, 1852.
11. Ryffel, J. H.: J. Physiol. **39**:29, 1910.

The presence of lactic acid as a sweat constituent and its occurrence there in concentrations much greater than are ordinarily found in blood, suggests that it is a product of chemical changes occurring in the sweat glands during secretion. Presumably it is an expression of the energy changes performed by the sweat glands in elaborating the typical secretion in a manner analogous to lactic acid formation during muscular work.

SUMMARY

1. The lactic acid contents of the blood, urine and sweat of patients suffering from arthritis and rheumatic disabilities has been determined and the results have been compared with the respective lactic acid contents from nonarthritic persons. The lactic acid was determined also in the synovial fluid from a case of joint effusion. In no case have we observed abnormal quantities of lactic acid in the arthritic patients. These findings therefore lend no support to the idea that arthritis, at least of the types studied, is caused or characterized by abnormal production or disposal of lactic acid.

2. Lactic acid has been found to be present in sweat in considerable quantities, making up about 50 per cent. of the organic acids of sweat. Its presence in this secretion may be referable to the chemical changes occurring in the sweat glands during their activity.

3. The benefit accruing to arthritic patients from sweating measures cannot, in the light of these results, be ascribed to the elimination of lactic acid in the sweat.

STUDIES ON THE METABOLISM OF OBESITY

III. THE SPECIFIC DYNAMIC ACTION OF FOOD *

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The first line of experimentation followed by us in our studies on the metabolism of obesity was directed toward the possible discovery of variations in the specific dynamic action of foods in persons of widely different body build. In 1921 one of us [1] reported our first experiments on the specific dynamic action of carbohydrate. No definite conclusions were drawn from this study. The work has subsequently been extended, until at the present time we are able to report the original series of experiments on carbohydrate utilization performed with the Benedict portable respiration apparatus, and a larger series done with the Tissot gasometer in which carbohydrate, protein and fat meals were studied.

When our work was first started, no very definite experimental data were available on variations in the specific dynamic action of foods. In the large series of cases studied by Benedict and Carpenter [2] no attention was paid in the protocols to the question of body weight. We attempted to correlate changes in the metabolism following food ingestion with original differences in the height-weight proportions of their subjects, but our analysis yielded nothing definite. In our first communication we, however, had expressed the opinion that, since up to that time no one had been able to explain obesity on the basis of variations in basal metabolism, a theoretical explanation might be found in one of two ways: (*a*) an unusual use of food stuffs, (*b*) variation in energy expense by different persons performing the same task. The conception that foodstuffs are not necessarily metabolized in the same way by all persons implied individual variations in the so-called specific

* From the Medical Clinic, the Gusta Morris Rothschild Fund and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research.

1. Strouse, Solomon: Carbohydrate Oxidation in Relation to Body Weight, American Soc. for Clinical Investigation, May 5, 1921, Chicago, American Medical Association, 1921.

2. Benedict, F. G., and Carpenter, T. M.: Food Ingestion and Energy Transformations, Publication No. 261, Carnegie Institution of Washington, 1918.

dynamic action of food. Other phases of the problem have been discussed in previous papers of this series.[3]

Although in the earlier literature numerous opinions are found concerning "slowing of metabolism" in obesity, incontrovertible experimental evidence is scant. The original report of Jaquet and Svenson,[4] of which we were not aware at the beginning of our research, contains a study of three cases of obesity, all of which showed normal basal metabolism. These three persons were given meat and mixed food, and the effect on the metabolism measured. The increases were compared with those found by Magnus-Levy [5] in 1893, and the authors state that "the increase in oxidation after food was significantly less and of shorter duration than with normals." In a matter so important for the understanding of a fundamental problem in metabolism, it seems fair to suggest in criticism of this work that the absence of normal standards of their own lessens the value of their conclusions.

No further experiments are available until 1915, when Means [6] studied the effect of glucose and of meat on the metabolism of one case of obesity. He concluded that "the specific dynamic action of protein is as great in this case of obesity as in normal individuals." Means used no normal controls and compared his results with the normal figures obtained by Gigon.[7] Of comparative interest perhaps is the contribution of Rabe and Plaut,[8] who found a diminished specific dynamic action of protein in a person accustomed to low nitrogen diet. Rolly [9] had a rare opportunity of studying specific dynamic action in obesity. One patient with tuberculosis of the testicle was studied before and after castration. His weight before castration was 56.7 kg. and some time after the operation was 86.1 kg. The specific dynamic action of protein was very high during the first period, and much lower when the patient became obese. This author also studied a woman with exophthalmic goiter who, after thyroidectomy and pregnancy, increased in weight from 57 kg. to 89.5 kg. The results were the same as in his first case. When, however, this woman was given thyroid

3. Strouse, Solomon; Wang, C. C., and Dye, Marie: Studies on the Metabolism of Obesity, Arch. Int. Med. **34**:275 (Sept.) 1924.

4. Jaquet, A., and Svenson, N.: Zur Kenntnis des Stoffwechsels fettsuchtiger Individuen, Ztschr. f. klin. Med. **41**:375, 1900.

5. Magnus-Levy, A.: Ueber die Grösse des respiratorischen Gaswechels unter dem einfluss der Nährungsaufnahme, Arch. f. d. ges. Physiol. **55**:1, 1893.

6. Means, James H.: Studies of the Basal Metabolism in Obesity and Pituitary Disease, J. M. Res. **32**:121, 1915.

7. Gigon, A.: Ueber den Einfluss der Nährungsaufnahme auf den Gaswechsel und Energieumsatz, Arch. f. d. ges. Physiol. **140**:509, 1911.

8. Rabe, F., and Plaut, R.: Zur Frage eiweissarmer Ernährung, Deutsch. Arch. f. klin. Med. **137**:187 (Aug.) 1921.

9. Rolly, F.: Zum Stoffwechsel bei der Fettsucht, Deutsch. med. Wchnschr. **47**:887 (Aug. 4) 1921.

extract therapeutically, the specific dynamic action of protein again rose. Although Rolly believes that his experiments suggest lowered specific dynamic action as a factor in producing obesity, it is evident that patients with tuberculosis and exophthalmic goiter are not suitable as "normal controls" and his experimental results cannot be accepted as conclusive.

In 1922 Plaut [10] published the results of an investigation of a large series of persons, normal and abnormal, on whom the specific dynamic action of a mixed meal was studied. This is the first published report on obesity of which we are aware, in which the investigator determined his own "normals." Plaut used the Benedict portable apparatus with standard technic. After basal metabolism was determined, a meal composed of 200 gm. of chopped beef, 50 gm. fat, 200 gm. bread and 500 c.c. coffee was given. In one hour the increase in oxygen consumption was again determined.

In well nourished persons Plaut found an increase of from 24 to 30 per cent. in the metabolism. Three stout, otherwise healthy persons showed the same increase. Forty-two abnormal persons were studied. Some of the author's results are found in Table 1.

TABLE 1.—*Specific Dynamic Action of Mixed Meal*

	Number of Cases	Percentage Increase
Normal persons	19	20 to 50
Constitutional obesity	12	4 to 29
Hypophyseal obesity	10	—3 to 18
"Constitutional thinness"	3	68, 40, 48 respectively

It will be seen that Plaut found a very definite lowering of the specific dynamic action of food in cases of obesity, and a very marked rise in the opposite type of persons, those with so-called "constitutional thinness." These experiments lasted only for one hour.

The following year Plaut [11] added twenty-six new cases to her first series with the same conclusions.

Liebesny [12] reported some excellent work on ten normal persons and sixty-one cases of obesity, complicated with endocrine disturbances. His test meal consisted of 200 gm. of roast veal and 100 gm. of bread. Basal metabolism was determined on each patient, and readings were taken one hour, one and a half hours, and sometimes two hours after the meal. He divided his cases into five groups on the basis of variation in metabolism and variation in specific dynamic action of protein,

10. Plaut, Rahel: Gaswechseluntersuchungen bei Fettsucht und Hypophysiserkrankungen, Deutsch. Arch. f. klin. Med. **139**:285 (May) 1922.

11. Plaut, Rahel: Gaswechseluntersuchungen bei Fettsucht, II, Mettheilung, Deutsch. Arch. f. klin. Med. **142**:266 (June) 1923.

12. Liebesny, P.: Die Zpezifisch-dynamische Eiweisswirkung, Biochem. Ztschr. **144**:308, 1924.

emphasizing particularly these conditions as affected by various types of endocrine disturbance. Liebesny reported the maximum rise in heat production as occurring at one and a half hours after the meal in most cases, and the percentage of increase as between 22.6 and 35.3 for normal persons. His conclusions for cases of obesity are found in Table 2.

TABLE 2.—*Liebesny's Conclusions for Cases of Obesity*

Class	Percentage Increase in Specific Dynamic Action *
Normal basal with normal specific dynamic action	24.9 to 36.3
Diminished basal with normal specific dynamic action	12.4 to 65.8
Approximately normal basal with diminished dynamic action	—1.2 to 15.2
Low basal with low specific dynamic action	1.7 to 11.7
Miscellaneous	6 cases varying widely

* These figures, taken from his tables, do not agree exactly with those in the body of his paper, also shown in the foregoing.

The reports of Plaut and Liebesny appeared after our initial report on the specific dynamic action of carbohydrates had been published and while our work on protein meals was in progress. On theoretical grounds we had hoped to find just such differences in the use of food by the obese as were reported by Plaut and Liebesny.

METHODS

A test meal was given in the morning immediately after the determination of the basal metabolism. The carbohydrate meal consisted of from 80 to 106 gm. of sucrose in the form of lemonade or of fondant flavored with mint. All patients except one had 100 gm. or slightly more of sucrose. Tests were taken following this meal at intervals of thirty minutes for one hour and sixty minutes for the two following hours. The fat meal was taken in the form of slightly sweetened ice cream made from 40 per cent. cream, the amount of fat varying from 55 to 129 gm. Tests were made at two-hour intervals for eight hours. The protein meal consisted of scraped beef, one or two glasses of milk, two slices of bread, and a small piece of butter, and contained from 32 to 66 gm. of protein. Tests were made at two-hour intervals and continued for eight hours. The amount of fat eaten by each subject was determined separately, but for carbohydrate and protein the figures were calculated from the tables given by Rose.[13]

Twenty-six tests were made on twelve obese subjects, using the Tissot gasometer apparatus. Of these twenty-six tests, eleven were made with the protein meal, eleven with the carbohydrate meal and four with the fat meal. These people varied in weight from 37 per cent. to

13. Rose, M. S.: A Laboratory Handbook for Dietetics, Revised Edition, 1922.

TABLE 3.—*Effect of Ingestion of Protein on Heat Production*

Subject	Sex*	Age, Years	Height, Cm.	Weight, Kg.	Weight, Per Cent. Difference from Normal	Protein Intake, Gm.	Calories per Square Meter per Hour: Basal, Calories	Basal, Per Cent. Difference from Basal	2 Hours After Meal, Calories	2 Hours After Meal, Per Cent. Difference from Basal	4 Hours After Meal, Calories	4 Hours After Meal, Per Cent. Difference from Basal	6 Hours After Meal, Calories	6 Hours After Meal, Per Cent. Difference from Basal	8 Hours After Meal, Calories	8 Hours After Meal, Per Cent. Difference from Basal
Overweight																
R. K.	♀	26	148.5	76.4	+45.1	42.6	42	+14.3	47	+10.0	43	+2.1	43	+1.4	45	+5.1
M. N.	♀	29	155	93 6	+72	36.8	30	−18.9	24	−20.1	23	−23.7	23	−24.8	32	+5.6
F. P.	♀	34	163.8	101.2	+64	65.3	39	+6.3	37	−5.6	39	+0.8	38	−1.6	42	+7.0
S. A.	♀	25	149.5	82.3	+56	44.8	37	−0.2	43	+15.1	39	+5.4	38	+1.8	36	−3.0
A. D.	♂	16	169.5	84.6	+37	66.4	41	−4.0	44	+6.5	46	+12.9	43	+4.3	43	+5.3
E. M.	♀	12½	160.5	97.8	+95	41.6	41	−11.6	42	+1.9	42	+1.6	39	−4.7	40	−3.5
E. M.	♀	12½	161.5	98.6	+94	44.5	..	.....	44	+9.8	43	+8.0	40	+0.1	40	+1.9
R. K.	♀	27	148.5	75.5	+43	46.8	36	−3.0	39	+9.8	42	+15.7	39	+9.1	37	+4.2
P.	♀	32	171.1	106.1	+58	39.1	34	−8.3	34	+2.1	35	+5.7	35	+3.5	33	−0.6
E. S.	♀	31	165	118.1	+91	53.8	48	+31.2	46	−4.3	42	−13.1	44	−7.7	44	−8.3
S.	♀	28	165	85.5	+42	32.0	35	−5.0	38	+9.0	36	+3.0	..	.....	..	.....
Average		25	.. ..	....	+63.3	46.6	38	0.1	40	+3.1	39	+1.7	38	−1.9	39	+1.3
Underweight																
M. A.	♀	25	166.2	47.3	−22	35.8	31	−16.2	39	+25.7	38	+22.9	35	+12.7	32	+3.1
M. A.	♀	25	166.2	47.3	−22	64.9	33	−11.9	36	+11.2	38	+17.3	38	+15.8	35	+8.3
B. McC.	♀	31	170	47.3	−28	43.9	36	−2.0	45	+26.1	46	+27.3	40	+11.6	41	+14.7
T.	♀	26	159.5	46.4	−23	54.5	41	+9.7	48	+19.2	47	+14.9	41	+1.7	40	−1.3
B. D.	♀	21	159.4	41.6	−26	78.3	38	+3.3	42	+10.2	43	+11.5	45	+17.2	44	+14.6
S. S.	♂	42	173.4	50.2	−31	58.2	36	−6.7	46	+28.9	46	+29.1	42	+16.0	42	+17.1
Average		28	....	....	−25.1	55.9	36	−4.0	43	+20.2	43	+20.5	42	+12.5	39	+9.4
Normal																
M. S.	♀	21	167.2	62.3	+3	53.5	34	−7.0	41	+18.8	43	+23.5	40	+16.6	39	+11.9
M. S.	♀	21	167.2	61.4	+2	61.1	36	−1.7	..	.....	43	+18.1	36	−0.4	35	−3.2
M. W.	♀	24	160	55.5	+1	53.1	42	+12.1	49	+17.4	48	+14.6	43	+3.4	44	+7.1
D. K.	♀	22	163.5	58.6	0	63.8	36	−2.8	43	+20.1	44	+21.0	42	+15.4	40	+11.8
E. T.	♀	22	163.8	54.2	−7	67.1	38	+5.0	43	+11.9	40	+6.1	37	−2.3	37	−4.1
J. S.	♀	21	161.0	57.7	+3	68.3	37	−0.9	43	+17.3	Coughed	.....	41	+11.7	39	+6.9
Average		22	.....	....	+0.5	61.2	37	+0.8	44	+17.1	44	+16.9	40	+7.2	39	+5.1

* In this column, ♀ indicates female, ♂ male.

140 per cent. above normal, and ranged in age from 12.5 to 34 years. Five otherwise normal underweight subjects, ranging in age from 21 to 42 years, and in weight from 22 to 31 per cent. below normal served for six tests with the protein meal, three with the carbohydrate meal, and two with the fat meal. The five normal subjects varied in weight from + 3 to — 7 per cent. from normal, and ranged in age from 21 to 24 years. Here there were six tests with the protein meal, three with the carbohydrate meal, and three with the fat meal.

COMMENT

The basal metabolism was discussed in a previous paper.[3] For convenience in handling the material this paper will be divided into three sections: (1) protein meal, (2) carbohydrate meal, and (3) fat meal.

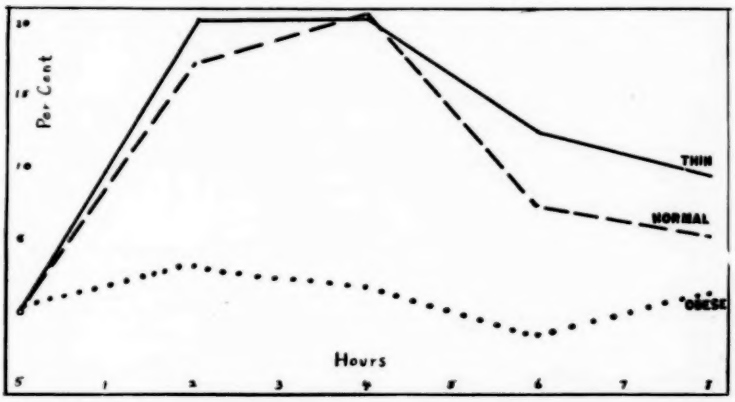

Chart 1.—Average percentage increase of energy production after the ingestion of food (high protein meal).

1. PROTEIN MEAL

Table 3 shows that the specific dynamic action of protein in obese people is very slight. In two cases there was no increase whatever above the basal, but a decreased heat production after the protein meal. In five of the remaining nine cases the highest value occurred two hours after the meal was taken, and in the rest four hours after ingestion. The highest figure shown at any time was 15.7 per cent. above the basal, and occurred after four hours. In only three of the forty-two readings were there values more than 10 per cent. higher than the basal. We had no cases with normal specific dynamic action as reported by Liebesny.[12] At the end of six hours all values were on the decline, and at the end of the eight hour period most of them had come very near

TABLE 4.—*Effect of Ingestion of Carbohydrate on Heat Production*

Subject	Sex*	Age, Years	Height, Cm.	Weight		Carbohydrate Intake, Gm.	Calories per Square Meter per Hour							
							Basal		½ Hour After Meal		1 Hour After Meal		2 Hours After Meal		3 Hours After Meal	
				Kg.	Per Cent. Difference from Normal		Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal
Overweight																
R. K.	♀	26	148.5	78.3	+48	106.2	47	+26.7	45	−3.5	51	+9.3	44	−5.7	42	−10.0
S. A.	♀	25	149.5	80	+51	106.4	41	+11.3	45	+8.7	43	+3.0	42	+0.9	42	+0.7
F. P.	♀	34	163.8	101.2	+68	103.4	34	−7.6	43	+26.1	40	+17.1	38	+11.2	37	+10.3
A. D.	♂	16	169.5	82.4	+33	104.3	38	−13.1	42	+10.0	41	+6.6	44	+15.9	39	+1.5
M. S.	♀	33	159.5	140	+141	103.4	38	+4.3	47	+22.3	43	+12.0	44	+14.4	42	+9.0
E. M.	♀	12½	161.5	100.9	+96	104.5	41	−11.9	50	+23.0	49	+20.3	42	+2.7	40	−3.3
E. S.	♀	34	165	117.8	+90	80.2	43	+18.0	47	+7.7	..	.....	45	+3.4	42	−3.0
S.	♀	28	165	83.7	+39	100	33	−10.0	43	+30.3	39	+18.2	39	+18.2	36	+9.1
S.	♀	28	165	84.1	+40	100	37	−3.8	39	+5.4	38	+2.7	36	−2.7	37	0.0
S.	♀	28	165	85.5	+42	100	39	+6.0	42	+7.7	40	+2.6	37	−5.1	36	−7.7
S.	♀	28	165	86.4	+44	100	37	−1.0	43	+16.2	38	+2.7	38	+2.7	36	−2.7
Average		27	.....	....	+63	100.8	39	+1.1	44	+14.0	42	+8.8	41	+5.1	39	+0.4
Underweight																
S. S.	♂	38	173.4	50.9	−30	100	37	−5.7	41	+10.8	41	+10.8	42	+13.5	41	+10.8
K.	♂	25	.....	61.4	−20	100	35	−11.0	39	+11.4	36	+2.9	40	+14.3	34	−2.9
B. D.	♀	21	158.8	41.6	−26	105.5	35	−5.2	41	+17.7	43	+23.6	44	+25.5	43	+22.7
Average		28	.....	....	−25.3	102.2	36	−7.3	40	+13.3	40	+12.4	42	+17.8	39	+10.2
Normal																
M. W.	♀	24	160	55.5	−1	104.9	39	−4.3	46	+17.9	45	+17.2	44	+14.4	41	+5.7
D. K.	♀	22	163.5	58.2	−1	104.4	33	−10.9	43	+29.6	42	+28.0	41	+23.1	36	+8.5
J. S.	♀	21	161.3	58.2	+2.3	106.0	37	+1.0	48	+28.2	46	+23.9	41	+9.6	37	−0.5
Average		22	.....	....	+0.3	105.1	36	−4.7	46	+25.2	44	+23.0	42	+15.7	38	+4.6

* In this column, ♀ indicates female, ♂ male.

the basal value. Individual differences were counterbalanced in the general average, which showed a value of only 3.1 per cent. above the basal at its highest point, two hours after the ingestion. At the end of the period the average value was within 1.3 per cent. of the basal.

Thin people showed a very different specific dynamic action of protein from that reported above. The least increase two hours after the meal was 10.2 per cent. and the maximum 28.9 per cent. over the basal. Four of the six cases gave the highest value four hours after the meal, and the greatest increase over the basal was 29.1 per cent. With the exception of one case the heat production was still above the basal at the end of eight hours. The average figures for the four two-hour periods following the meal were as follows: 20.2, 20.5, 12.5 and 9.4

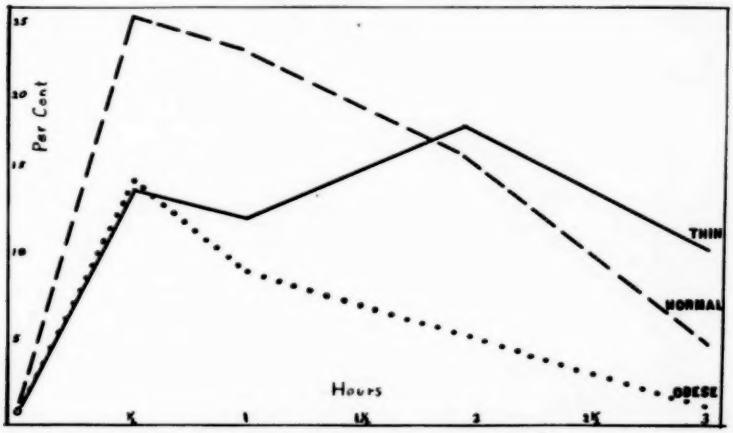

Chart 2.—Average percentage increase of energy production after the ingestion of food (high carbohydrate meal).

per cent. above the basal. It is interesting to note that the highest specific dynamic action was shown by the subject who was most underweight.

Normal people (Table 3), on the whole, showed a reaction approximately like that of thin people. Two hours after the meal the increase ranged from 11.9 to 20.1 per cent. above the basal, with an average of 17.1 per cent. The corresponding figures for the four-hour period were from 6.1 to 23.5 per cent., with an average of 16.7 per cent. above the basal. After this period there was a steady decline until at the end of eight hours the average was still 5.1 per cent. above the basal. The changes described are shown graphically in Chart 1.

There is apparently no relation between the amount of protein intake and the specific dynamic action in obese, thin or normal subjects.

TABLE 5.—*Effect of Ingestion of Fat on Heat Production*

Subject	Sex*	Age, Years	Height, Cm.	Weight		Fat Intake, Gm.	Calories per Square Meter per Hour							
							Basal		2 Hours After Meal		4 Hours After Meal		6 Hours After Meal		8 Hours After Meal	
				Kg.	Per Cent. Difference from Normal		Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal	Calories	Per Cent. Difference from Basal
Overweight																
M. S.	♀	33	159.5	131.3	+136	54.7	42	+16.4	42	−1.8	44	+3.7	42	−0.9	39	−7.9
A. D.	♂	16	169.5	83.7	+35	109.0	37	−15.1	41	+11.2	42	+14.2	40	+8.7	42	+15.6
A. D.	♂	16	.....	87.7	+46	82.8	39	−8.4	42	+5.5	42	+7.2	42	+7.0	43	+8.6
E. M.	♀	12½	161.5	98.6	+94	129.1	42	−9.6	40	−3.8	41	−2.1	41	−2.7	40	−4.3
Average		20	.....	....	+78	93.9	40	−4.2	41	+2.8	42	+5.8	41	+3.0	41	+3.0
Underweight																
B. D.	♀	21	159.1	41.5	−26	50.7	35	−4.2	38	+8.3	37	+4.3	41	+14.6	39	+8.6
S. S.	♂	42	173.4	50.2	−31	53.3	38	+8.4	43	+12.6	40	+4.7	40	+5.4	41	+8.3
Average		34	.....	....	−28.5	52.0	37	+2.1	41	+10.5	39	+4.5	41	+10.0	40	+8.5
Normal																
M. W.	♀	24	160	55	−2	79.8	39	+4.9	41	+5.7	40	+2.7	43	+9.5	41	+6.7
D. K.	♀	22	163.5	58.2	+2	84.5	35	−4.4	38	+6.1	37	+4.9	37	+8.3	36	+0.8
J. S.	♀	21	160.3	57.2	+2	55.0	38	+2.4	41	+8.5	39	+3.1	40	+6.1	36	−5.9
Average		22	.....	....	+0.7	73.1	37	+1.0	40	+6.8	39	+3.6	40	+6.3	38	+0.5

* In this column, ♀ indicates female, ♂ male

The thin subject showing the greatest specific dynamic action, that is, 29.1 per cent. above the basal at the end of four hours, received only 58.2 gm. of protein, whereas another subject in the same group ate 78.3 gm. of protein and had a maximum value of 17.2 per cent.

2. CARBOHYDRATE MEAL

The specific dynamic action of carbohydrate is not so marked in the three groups as that of protein (Table 4). With the exception of two cases, the greatest increase in heat production in obese people occurred one-half hour after the meal. The maximum increase was 30.3 per cent. over the basal and the minimum 5.4 per cent.; the average increase in this period was 14 per cent. From this time the figures decreased steadily to the end of the three-hour period, when

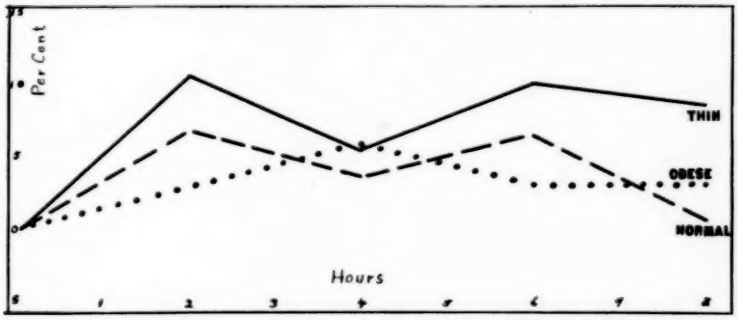

Chart 3.—Average percentage increase of energy production after the ingestion of food (high fat meal).

half of the cases had returned to the basal value or lower. The average value at this time was only 0.4 per cent. higher than the basal. The three thin subjects showed the highest values at the end of two hours, and two remained high after three hours. The maximum values ran from 13.5 to 25.5 per cent. above the basal, with an average of 17.8 per cent. At the end of the period the highest was still 22.7 per cent. above the basal, but one case had dropped to 2.9 per cent. below. The normal subjects showed the greatest increase of heat production, with a curve similar in general outline to that of the obese people. The highest values, at the end of thirty minutes, ranged from 17.9 to 29.6 per cent. above the basal, with an average of 25.2 per cent. At the end of the period the lowest attained were from 0.5 per cent. below to 8.5 per cent. above the basal, with an average of 4.6 per cent. above.

The obese person does not always react to the carbohydrate meal in a characteristic manner, but the average curves show a tendency toward lessened specific dynamic action of carbohydrate (Chart 2).

3. FAT MEAL

With the ingestion of fat there was a slight increase in heat production in all cases (Table 5). This may have been due to the small amount of carbohydrate and protein contained in the food. The increase was slightly higher in thin people. In the obese subjects the maximum value occurred at the end of four hours, and in both normal and thin people there were two peaks, one at the end of two hours, and the other at the end of six hours. Only one normal subject showed but one peak, and that after two hours. The average maximum increase for obese people was 5.8 per cent. above the basal, for thin people 10.5 per cent., and for normal subjects 6.8 per cent. (Chart 3).

SUMMARY

Twenty-six tests were made on twelve obese subjects, using protein, carbohydrate and fat meals; eleven tests on five thin people, and twelve tests on five normal subjects.

Protein showed a very slight specific dynamic action in obese people. Thin people, on the contrary, showed a very high specific dynamic action of protein. Normal subjects followed much the same course as the thin people, but to a less degree.

Normal subjects had the most marked specific dynamic action of carbohydrate. Although obese persons did not react in a uniform manner to carbohydrate intake, the average for the group indicated a lessened specific dynamic action of carbohydrate. The reaction in the three groups did not show so great a degree of difference as occurred after the protein meal.

There was very little, if any, evidence of specific dynamic action of fat.

Book Review

TEXTBOOK OF PHARMACOLOGY AND THERAPEUTICS, OR THE ACTION OF DRUGS IN HEALTH AND DISEASE. By ARTHUR R. CUSHNY, Professor of Pharmacology and Materia Medica, University of Edinburgh. Ed. 8, thoroughly revised. Philadelphia, Lea and Febiger. Price, $6.00.

A new revised edition of this standard textbook will be appreciated by those interested in the progress of materia medica. As evidence of the progress of therapeutics, the author states that the space allotted to remedies for specific diseases is five times as great as in the 1903 edition.

The chapter on digitalis and cinchona bases has been brought up to date. A new chapter on histamin action and the relation of symptoms produced by it to anaphylaxis and a chapter on insulin and vitamins have been added.

As in previous editions, the chapter on digitalis is very complete, both in the physiologic action of the drug, its clinical use and evidence of intoxication. Vitamins are discussed in a lucid and concise manner. The presence of vitamin in cod liver oil explains its usefulness in malnutrition and rickets.

A paragraph is devoted to quinidin in auricular fibrillation and the manner of action compared with that of digitalis.

In the chapters on organotherapy, the suprarenal, pituitary, pancreas and thyroid extracts are discussed in detail. With true Scotch caution, which appeals to all those interested in the progress of therapeutics, he dismisses the remainder of organotherapy with the following sentence: "The theory on which many of these have been involved shows little advance on the belief of the savage that the courage of the lion may be acquired by eating the animal's heart, and the clinical observations which have been cited to support their use have usually been of an equally primitive order."

In spite of the additions made to certain chapters there is no increase in the size of the volume, as the descriptions of less reliable drugs have been curtailed. The author states that a textbook of this character must point out the worthlessness of certain preparations, in addition to describing the virtues of established remedies.

As a teacher he confines his discussion to relatively few important drugs, rather than confuse the student with the supposed value of a large number of practically worthless agents. This same sane method is adopted in his textbook, and this accounts in a large measure for its being accepted as a standard work, suitable for students' or practitioners' use.